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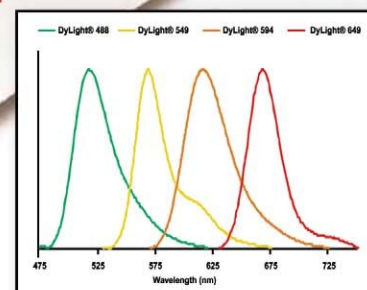
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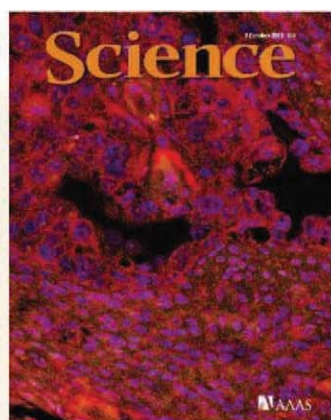
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COVER

Confocal microscopy image of human ovarian clear cell carcinoma tissue. Cancerous epithelial cells are stained bright red (top half of image) and are bordered by supporting stromal cells (bottom and upper right corner). Cell nuclei are stained purple. Genetic analysis by Jones *et al.* suggests that aberrant chromatin remodeling contributes to the pathogenesis of this cancer. See page 228.

Image: Ie-Ming Shih and Bin Guan

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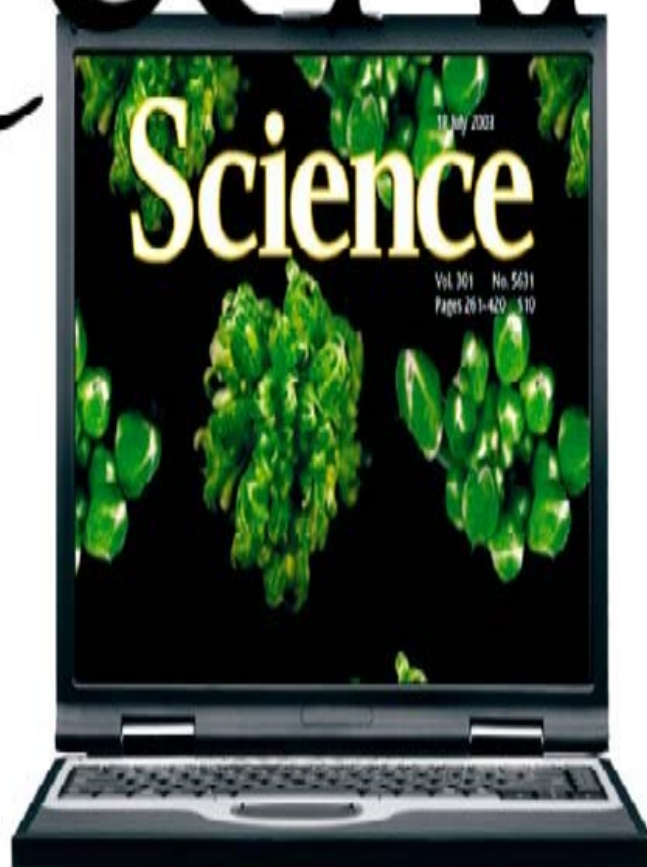
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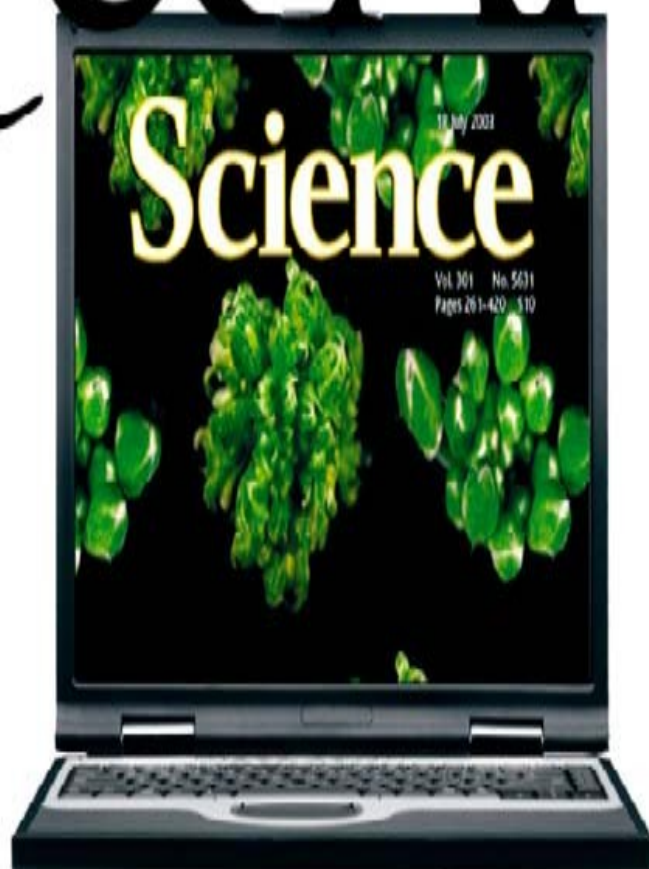
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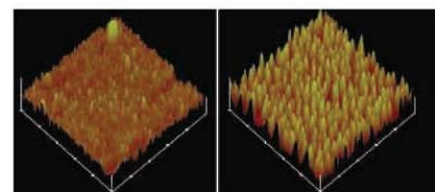
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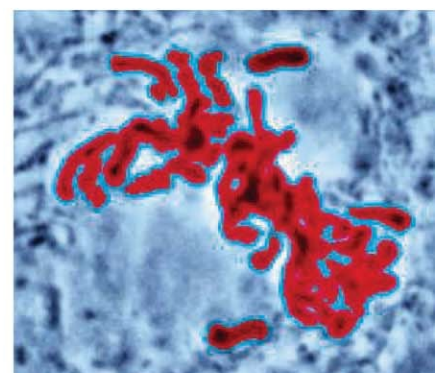
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V. S. Bajaj et al.

A magnetic resonance imaging system allows
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10.1126/science.1192313

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Mutations causing a rare skin disease reveal
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**Comment on "Climate, Critters, and Cetaceans:
Cenozoic Drivers of the Evolution of Modern
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N. D. Fyenson et al.

Full text at [www.sciencemag.org/cgi/content/
full/330/6001/178-a](http://www.sciencemag.org/cgi/content/full/330/6001/178-a)

**Response to Comment on "Climate, Critters,
and Cetaceans: Cenozoic Drivers of the
Evolution of Modern Whales"**

F. G. Marx and M. D. Uhen

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A. ElAli and D. M. Hermann

PODCAST

D. M. Hermann and A. M. VanHook

Inhibition of apolipoprotein E signaling is a
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**RESEARCH ARTICLE: Nonsynaptic Communication
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SCIENCE CAREERS

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Editorial: Happy 15th Birthday to Us

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Top Ten Tips for Mentors

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Finding a Future in Management Consulting

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When she couldn't find a professorship in her
native Portugal, medical physicist Francisca Leite
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**COMMENTARY: Opening Up to
Precompetitive Collaboration**

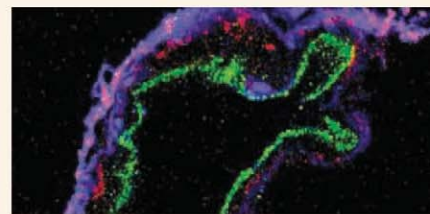
J. S. Altshuler et al.

Increasing biomedical R&D efficiency and
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**RESEARCH ARTICLE: Effects of AIN457, a Fully
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An antibody to a proinflammatory factor may be
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human diseases.

**RESEARCH ARTICLE: PGC-1 α , A Potential
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in Parkinson's Disease**

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A master controller of genes for energy regulation
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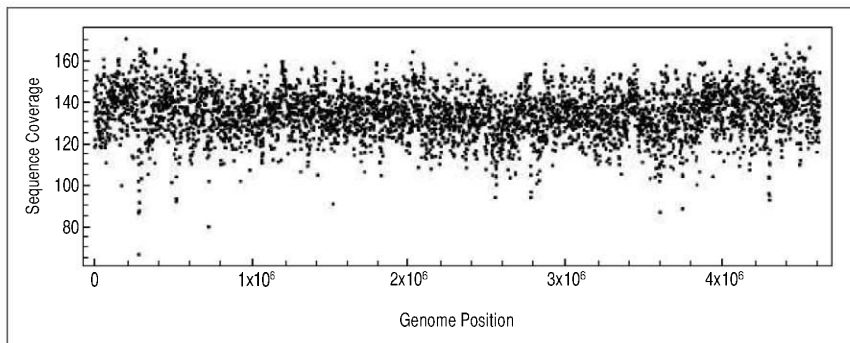
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<< Diving into Deep Water

The Deepwater Horizon oil spill in the Gulf of Mexico was one of the largest oil spills on record. Its setting at the bottom of the sea floor posed an unanticipated risk as substantial amounts of hydrocarbons leaked into the deepwater column. Three separate cruises identified and sampled deep underwater hydrocarbon plumes that existed in May and June, 2010—before the well head was ultimately sealed. **Camilli *et al.*** (p. 201; published online 19 August) used an automated underwater vehicle to assess the dimensions of a stabilized, diffuse underwater plume of oil that was 22 miles long and estimated the daily quantity of oil released from the well, based on the concentration and dimensions of the plume. **Hazen *et al.*** (p. 204; published online 26 August) also observed an underwater plume at the same depth and found that hydrocarbon-degrading bacteria were enriched in the plume and were breaking down some parts of the oil. Finally, **Valentine *et al.*** (p. 208; published online 16 September) found that natural gas, including propane and ethane, were also present in hydrocarbon plumes. These gases were broken down quickly by bacteria, but primed the system for biodegradation of larger hydrocarbons, including those comprising the leaking crude oil. Differences were observed in dissolved oxygen levels in the plumes (a proxy for bacterial respiration), which may reflect differences in the location of sampling or the aging of the plumes.

Nitrogen's Past and Future

Microorganisms have been controlling Earth's nitrogen cycle since life originated. With life evolving around it, nitrogen became both an essential nutrient and a major regulator of climate. **Canfield *et al.*** (p. 192) review the major changes in the nitrogen cycle throughout Earth's history. Most of the time, perturbations typically coincided with the evolution of new metabolic pathways in various Bacteria or Archaea. The last century, however, has seen humans push the biological nitrogen cycle into a new stage altogether. The addition of large quantities of fixed nitrogen to crops in the form of fertilizer chokes out aquatic life that relies on runoff and adds significant amounts of N_2O —a potent greenhouse gas—to the atmosphere. Although microorganisms may one day restore balance to the nitrogen cycle that they helped shape for billions of years, humans must modify their behavior or risk causing irreversible changes to life on Earth.

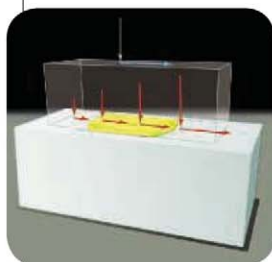
Feeling the Pinch

Nanoparticles exhibit many different properties compared to their larger counterparts, but how the stability of certain phases relative to others is affected by a change in particle size is often unclear. Using a thermodynamic probe sensitive to nanoparticle surfaces, **Navrotsky *et al.*** (p. 199) show that surface energy strongly influences the stability of some metal oxides relative to others. For example, nanoparticles of CoO , which are stable at larger sizes, are only stable over a very narrow range of conditions due their high surface energies. Cobalt oxides are catalysts

that may one day promote the cost-effective generation of hydrogen from water, but nanoparticles in soils and biological systems—including iron and manganese oxides—also feel the pinch of surface energy on their range of stability.

Slip Sliding Away

The initiation of frictional motion, or slip, along ideal surfaces typically behaves as predicted by rupture models. When stress heterogeneities—



similar to irregularities in fault zones in Earth's crust—are introduced, rupture propagation speeds are not as well constrained by models. To improve understanding of slip behavior, **Ben-David *et al.***

(p. 211; see the Perspective by **Zapperi**) measured rupture speeds and stress profiles along an extended frictional interface between two polymer blocks. The experiments revealed a slow mode of slip that occurs when the local ratio of shear to normal stress is sufficiently low. The selection and arrest of three distinct modes of rupture depended on the value of the local stress ratio.

Polymer Templating for Metals

Polymer templating has been used to fabricate a wide range of ordered materials, both due to the ability to pattern the polymers easily over

a large area and their facile removal. However, the process is somewhat limited to the incorporation of materials that will flow easily into the templated areas. **Arora *et al.*** (p. 214) show that current techniques can be extended to the patterning of metals, through guided epitaxial growth. An excimer laser was used to control the flow of material into patterned templates formed from block copolymers.

Polysaccharide Breakdown

One of the current challenges in the biofuels industry is achieving efficient bioconversion of complex polysaccharides like cellulose and chitin. Recently, chitin-binding proteins (CBPs) have been identified that potentiate chitin hydrolysis. Now, **Vaaje-Kolstad *et al.*** (p. 219) show that a CBP from the chitinolytic bacterium *Serratia marcescens* appears to catalyze an oxygenase reaction on the surface of crystallized chitin, leading to chain breakage and generating oxidized ends that can be degraded by chitinases. A structurally similar enzyme, GH61, may play a similar role in the degradation of cellulose.

Economic Benefits of Bt Maize

Maize containing a transgenically expressed toxin originating from *Bacillus thuringiensis* (Bt maize) is planted across the United States to combat insect herbivory. Non-Bt Maize is also planted alongside Bt maize fields to provide refuges for the insects, which helps to prevent

Continued on page 149

DEEPWATER
HORIZON

GULF OF MEXICO OIL SPILL

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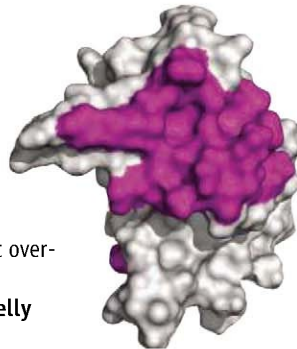
resistance to Bt maize from evolving. **Hutchison *et al.*** (p. 222; see the Perspective by **Tabashnik**) analyzed how Bt maize affected the economic impact of the European corn borer moth in the midwestern United States, as well as its population dynamics. Larval density, a predictor of corn borer population size, has dropped in correlation with the percentage of Bt maize planted. In the highest Bt maize producing state, the positive effects of Bt maize in controlling insect herbivore populations extended to non-Bt maize. Furthermore, the decrease in insect populations demonstrated an overall economic benefit outweighing the overall extra costs associated with planting Bt maize.

Freezing Tolerance Explained

Freezing temperatures exact a toll on plant cells through various mechanisms, including disruption of water balances as ice crystals form. Cellular and organelle lipid bilayers are also put under stress. **Moellering *et al.*** (p. 226, published online 26 August; see the Perspective by **Browse**) analyzed the function of a protein in the model plant, *Arabidopsis thaliana* that, when disrupted, leaves plants more susceptible to damage by freezing. The protein, SENSITIVE TO FREEZING 2 (SFR2), shifts and swaps lipid headgroups, altering the chemistry of the chloroplast lipid bilayer membranes to stabilize them during freezing.

Location, Location, Location

Cell division is orchestrated by a complex signaling pathway that ensures the correct segregation of newly replicated chromosomes to the two daughter cells. The pathway is controlled in part by restricting the activity of critical regulators to specific subcellular locations. For example, the chromosomal passenger complex (CPC) is recruited to chromosomes during mitosis where it oversees kinetochore activity and cytokinesis (see Perspective by **Musacchio**). **Wang *et al.*** (p. 231, published online 12 August), **Kelly *et al.*** (p. 235, published online 12 August), and **Yamagishi *et al.*** (p. 239) now show that the phosphorylation of the chromatin protein, histone H3, acts to bring the CPC to chromosomes, thereby activating its aurora B kinase subunit. The Survivin subunit of CPC binds specifically to phosphorylated H3, with the phosphorylation at centromeres being carried out by the mitosis-specific kinase, haspin. Furthermore, Bub1 phosphorylation of histone H2A recruits shugoshin, a centromeric CPC adapter. Thus, these two histone marks in combination define the inner centromere.



Remodeling Gone Awry

The identification of genes that are mutated at high frequency in human tumors can provide important clues to the molecular pathways that drive tumor growth, which in turn can potentially lead to more effective therapies. **Jones *et al.*** (p. 228, published online 9 September; see the cover) looked for such mutations in ovarian clear cell carcinoma, a rare but particularly lethal form of ovarian cancer. Of 42 tumors examined, 57% were found to harbor inactivating mutations in *ARID1A*, a gene coding for a subunit of the SWI/SNF chromatin remodeling complex, which functions as a master regulator of transcription factor action and gene expression. Thus, proteins associated with the epigenetic control of gene expression can contribute to the development of human cancer.

Web of Parasite Interactions

We live under constant assault from a variety of pathogens. Pathogen exposure will be more or less harmful depending on host factors, including immune status, and, as **Telfer *et al.*** (p. 243; see the Perspective by **Lafferty**) point out, the presence of co-infecting pathogens. In a time-series study of wild voles and four pathogens, co-infection had a larger effect on disease than any other factor. For example, infection with cowpox virus increased susceptibility and prolonged bacterial co-infections. Conversely, an ongoing infection with the bacterium *Anaplasma* reduced the rodents' susceptibility to the protozoan *Babesia*. In turn, chronic infection with *Babesia* limited susceptibility to the bacterium *Bartonella*.

CREDIT: KELLY ET AL.

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S. James Gates Jr. is the John S. Toll Professor of Physics and director of the Center for String and Particle Theory at the University of Maryland, College Park, MD, and a member of PCAST. E-mail: gates@wam.umd.edu.

Prepare and Inspire

A FEW WEEKS AGO, WE AND OUR COLLEAGUES ON THE PRESIDENT'S COUNCIL OF ADVISORS ON Science and Technology (PCAST) presented President Obama with the report *Prepare and Inspire: K-12 Education in Science, Technology, Engineering and Math (STEM) Education for America's Future*.^{*} It advocates a two-pronged strategy: To prepare all students to use STEM in their personal and professional lives, and to inspire them to learn STEM subjects and pursue STEM careers. It also provides a roadmap of programs that the federal government must seriously consider if the United States is to remain a vibrant and innovative society.

More than 25 years ago, the report *A Nation at Risk: The Imperative For Educational Reform* sounded an alarm about America's K-12 (early education through precollege) educational system. Subsequent studies have highlighted the poor performance of the United States in STEM education, as assessed by comparative student achievement. This is of special concern because it is science and technology that propelled most of the increase in U.S. per capita income in the past century.

The good news is that the United States has begun to respond. A bipartisan consensus has emerged about the importance of educational accountability, and last year, approximately \$4.4 billion dollars was committed in the Department of Education's Race to the Top program to support educational reform. The National Academy of Sciences has distilled research about how students learn math and science, providing a base of knowledge for moving forward. This year, 36 states and the District of Columbia have adopted common mathematics education standards, and shared science standards are under discussion.

Our PCAST report includes five steps that the federal government should take to enhance the nation's future well-being. First, existing high-quality programs should be scaled up to prepare 100,000 STEM teachers over the next decade. Second, to recognize excellence in STEM teaching, a STEM Master Teacher Corps should be created to award salary supplements and resources to support professional activities and networking. Third, a new federal entity, ARPA-Ed (Advanced Research Projects Agency-Education), should develop shared technology platforms and digital instructional materials to help teachers and students harness the potential of technology. Fourth, the government should help states increase the number of STEM-focused schools from 100 to at least 1000 over the next decade, in diverse communities and at multiple grade levels. Finally, a coordinated initiative (called INSPIRE) would give students opportunities to form connections with STEM through after-school programs.

The federal government has historically lacked a coordinated strategy and strong leadership on STEM education. Therefore, the PCAST report calls for a new partnership between the National Science Foundation and the Department of Education, with greater staff capacity at each agency. It also recommends increasing leadership, capacity, and coordination among the many federal agencies with disconnected programs for K-12 STEM education.

Some of these recommendations may require new authorities and/or appropriations.[†] But many federal actions can begin immediately, including scaling up STEM teacher preparation programs, directing existing funds to establish STEM-focused schools, and laying the groundwork for ARPA-Ed and a STEM Master Teacher Corps. STEM professionals must also act immediately. They should show unwavering support for the shared science education standards and encourage senators and representatives to enact programs in the PCAST report through appropriate legislation. STEM professionals must volunteer to help schools form robust connections to the STEM communities in academia, the private sector, and nonprofit organizations. It is important that the United States hold its leaders accountable for rising to the challenge of preparing its citizens for a competitive future, but everyone in the STEM community must rise to meet this challenge as well.

— Eric S. Lander and S. James Gates Jr.



10.1126/science.1198062

^{*}www.whitehouse.gov/sites/default/files/microsites/ostp/pcast-stemed-report.pdf. [†]J. Mervis, *Science* **329**, 1582 (2010).



Cyanomitra oritis feeding on *Impatiens sakeriana*.

ECOLOGY

Best Feed Forward

New World hummingbirds and Old World sunbirds are both adapted for feeding on nectar. They share specialized traits, such as an elongated bill and a small body size. Hummingbirds, however, have become famous for their ability to hover indefinitely in front of flowers, whereas sunbirds seem less able or inclined to do so. Following on a recent study that demonstrated that African sunbirds can hover when challenged to feed from an invasive, hummingbird-pollinated New World plant (*Nicotiana glauca*), Janeček *et al.* have examined the pollination system of the native African plant *Impatiens sakeriana*, which displays a suite of characteristics common to plants that attract hovering birds: red flowers, a long flower spur, high nectar production, and an absence of nearby perching structures as is usually observed in sunbird-pollinated plants. The reproductive ecology of this plant has been a conundrum. It had been hypothesized that it evolved to be pollinated by a now-extinct hovering bird; in this species' absence, it would have to be either self- or insect-pollinated. The authors used exclusion and germination experiments along with observations to demonstrate that these plants are not capable of self-pollination and are not pollinated by insects. Further, they showed that two species of African sunbirds (*Cyanomitra oritis* and *Cinnyris reichenowi*) will both perch and hover at *I. sakeriana* flowers. Nevertheless, *C. reichenowi*, when perching, pierces the sides of flowers to drink the nectar, resulting in lower seed production. Therefore, the authors suggest that the distinctive flower morphology of *I. sakeriana* has evolved in order to induce birds to hover, instead of perch, and thus to enforce a fair trade of nectar for pollination. — SNV

Oikos **119**, 10.1111/j.1600-0706.2010.08612.x (2010).

PHYSICS

A Fertile Majorana Habitat

Most species of (near-) elementary particles (such as electrons and protons) have their own antiparticle partners with which they annihilate in a burst of gamma rays. Usually the antiparticle is distinct from the particle. An exotic exception to that rule is the spin-1/2 Majorana fermion, which is its own antiparticle. Although predicted more than 70 years ago, Majorana fermions are yet to be observed, with neutrinos and dark matter WIMPs being the prime candidates. Recently, another possible setting for Majoranas emerged in solid-state materials such as certain topological insulators (TIs) that have a linear electron dispersion but are also superconducting. On doping the TI Bi₂Se₃ with copper, a superconducting state was observed; however, calculations indicated that such doping would destroy the conditions necessary to support the Majorana state. Now, Wray *et al.* find in their angle-resolved photoemission experiments that this is not the case, raising a realistic possibility of a first observation of a Majorana fermion. — JS

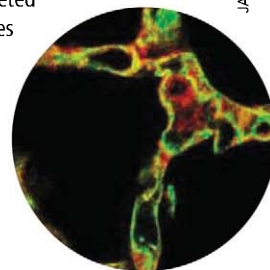
Nat. Phys. **6**, 10.1038/nphys1762 (2010).

CELL BIOLOGY

Keeping Things Clear

Pneumonia is a serious inflammatory lung disease commonly caused by bacterial infection. It is a major cause of death in all age groups and particularly in those who are chronically ill, and further insight into its pathogenesis may promote the development of new therapeutic approaches. Ray *et al.* have discovered an alternative mechanism underlying the pathobiology of pneumonia caused by bacterial infection. Cardiolipin is a phospholipid that mitochondrial membranes are rich in. It is a minor component of pulmonary surfactant, which is secreted into the airways and lines the alveolar surface of the lungs, reducing

Atp8b1 (red) at the cell surface (green) in a mouse lung.



surface tension and enabling proper lung function. Higher concentrations of cardiolipin are found in animal models of lung injury, suggesting that its regulation is important in disease pathogenesis. After discovering higher levels of cardiolipin in fluid isolated from the lungs of pneumonia patients, the authors uncovered a critical role for cardiolipin in surfactant activity and in lung structure and

JANEČEK, RAY ET AL., *NAT. MED.* **16**, 10.1038/NM.2213 (2010)

CREDITS (TOP TO BOTTOM): STEPAN

function in mice. The extracellular levels of cardiolipin are regulated by the lipid transporter Atp8b1, which imports phospholipids across the plasma membrane. Mutants of Atp8b1 are found in a familial disorder associated with an increased risk of pneumonia. Indeed, by overexpression and loss-of-function studies in cultured cells and in mice, the authors established a critical role for Atp8b1. These results suggest that the clearance of cardiolipin from the lungs may be of therapeutic benefit for treating pneumonia caused by bacteria, and thus could help to alleviate the current dependence on broad-spectrum antibiotics, which has led to the emergence of dangerous drug-resistant strains. — HP

Nat. Med. **16**, 10.1038/nm.2213 (2010).

CHEMISTRY

Golden Selection

Gold's widespread use in jewelry design and dentistry is due in large part to its remarkable resistance to chemical oxidation. There is in fact a fairly straightforward means of dissolving the metal—a concentrated combination of nitric and hydrochloric acids that's been in use for hundreds of years—but this solvent mixture is so generally corrosive that rings and fillings wouldn't be the primary worry if some happened to spill onto your fingers and teeth. Of more commercial concern is the acid mixture's failure to discriminate between gold and platinum, which hampers catalyst-recycling protocols. Lin *et al.* have discovered that a different, nonaqueous solvent mixture—thionyl chloride and pyridine—can also dissolve gold quite effectively but leaves platinum fully intact. The process is clearly oxidative, although the exact product is somewhat uncertain; x-ray photoelectron spectroscopy implicates the formation of trivalent gold species, and a salt of the tetrachloride anion $[\text{AuCl}_4]^-$ precipitates after several months. A number of other aromatic amines, as well as dimethyl formamide, can substitute for the pyridine. — JSY

Angew. Chem. Int. Ed. **49**, 10.1002/anie.201001244 (2010).

ECOLOGY

Shedding Dead Leaves

Historically, New Zealand had flourished without terrestrial mammalian fauna until the relatively recent arrival of humans. As a consequence, many plants have evolved in the absence of selective pressures generally attributed to mammalian lineages. One such example is the grass family, which is believed to have coevolved elsewhere



with grazing ungulates, such as modern-day horses. Many grasses show specific adaptations to grazing, including the retention of old leaves, which makes the live leaves less readily accessible to grazing mammals. Antonelli *et al.* demonstrate that

many members of the danthonioid group of grasses of New Zealand (such as *Rythidosperma pulchrum*, above) have gained the ability to lose dead leaves, a process known as abscission, which is a relatively rare trait that occurs in only ~3% of graminoids worldwide and that allows plants to generate more biomass. The authors attribute this pattern to the lack of selective grazing pressures by mammals and demonstrate how this trait is correlated with species radiations in New Zealand but not in other endemic floras. These data suggest that release from a selective pressure can lead to species radiations and contribute to the uniqueness of biotas. — LMZ

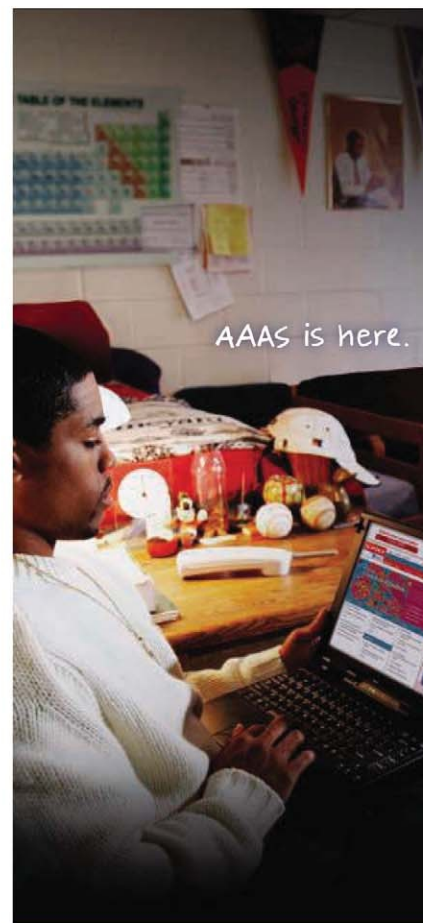
Proc. R. Soc. London Ser. B **277**, 10.1098/rspb.2010.1145 (2010).

MICROBIOLOGY

Stressed by Heavy Metal

When an ecosystem is stressed, do the more tolerant species benefit or do all species decline? In natural microbial communities, examples of both responses occur, but comparisons across field sites and experiments are difficult. In a relatively controlled natural setting, Gough and Stahl studied the stress response to metals in anoxic sediments of a eutrophic lake that are loaded with high but variable levels of metal contaminants from a nearby zinc smelter. By using a broad sequencing technique to identify dominant microbial sequences at the domain level, they found that across a range of metal concentrations, the communities were quite similar, and except in a few instances, the abundance and diversity of bacteria, archaea, and eukaryotes were uncorrelated with metal concentrations. In a notable exception within the archaeal fraction, cold-adapted Crenarchaeota were strongly correlated with high levels of zinc, arsenic, and copper. Although little is known about these freshwater anaerobes—even their metabolic role in the microbial communities is unclear—they appear to have a competitive advantage in their response to metal stress over other archaeal clades, including more common methanogens. — NW

ISME J. **4**, 10.1038/ismej.2010.132 (2010).



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Jonathan D. Cohen, *Princeton Univ.*

Andrew Cossins, *Univ. of Liverpool*

Robert H. Crabtree, *Yale Univ.*

Wolfgang Cramer, *Potsdam Inst. for Climate Impact Research*

F. Fleming Crim, *Univ. of Wisconsin*

Jeff L. Dangl, *Univ. of North Carolina*

Stanislav Dehaene, *Collège de France*

Edward DeLong, *MIT*

Emmanouil T. Dermatzakis, *Univ. of Geneva Medical School*

Robert Desimone, *MIT*

Claude Desplan, *New York Univ.*

Ap Dijksterhuis, *Radboud Univ. of Nijmegen*

Dennis Discher, *Univ. of Pennsylvania*

Scott C. Donay, *Woods Hole Oceanographic Inst.*

Jennifer A. Doudna, *Univ. of California, Berkeley*

Julian Downward, *Cancer Research UK*

Bruce Dunn, *Univ. of California, Los Angeles*

Christopher Dye, *WHO*

Michael B. Elowitz, *Calif. Inst. of Technology*

Gerhard Ertl, *Fritz-Haber-Institut, Berlin*

Mark Estelle, *Indiana Univ.*

Barry Everitt, *Univ. of Cambridge*

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October 15



ADVANCING SCIENCE. SERVING SOCIETY



Three Q's

Even among the 23 unusual individuals awarded no-strings-attached \$500,000 "genius grants" by the MacArthur Foundation last week, **John Dabiri** stands out. A biophysicist at the California Institute of Technology in Pasadena, Dabiri studies the fluid mechanics of how jellyfish swim. *Science* spoke with him about his research, which he says could be applied to systems such as the beating heart or even wind farms.

Q: Why jellyfish?

What I'm primarily interested in is the fact that they're so simple. Other, higher organisms have greater neural capacity. These are quite simple, but by using fluid mechanics they're able to achieve complex behaviors. We've been looking at vortex rings, which are like smoke rings you'd blow with a cigar. It turns out that by creating those rings they're able to move through the water more efficiently than they otherwise could.

Q: What can studying them tell us about the heart?

Not all jellyfish are created equal. Some are highly efficient; some are fast but less efficient. ... These properties show up in the types of rings they form. Similarly, we can look at the flow in the left ventricle in the heart, which also forms these vortex rings, and correlate shape and size to various performance levels in the heart.

Q: Any plans yet for the money?

I'm going to take swimming lessons. We go into the ocean ... and use combinations of different laser techniques ... to infer the water motion and measure the velocity and forces being generated. ... Since I never learned how to swim, the students and postdocs are the ones who take the measurements in the water. ... I'd like to join them.

Disgraced, Dismissed, But Still Herr Doktor

Jan Hendrik Schön may have ruined his scientific career, but he can keep his doctorate, a judge in Freiburg, Germany, decided last week. In 2002, the physicist touched off one of the biggest scandals to rock physics when it became clear that he had fabricated data in at least 17 papers on the electronic properties of organic materials (*Science*, 4 October 2002, p. 30). After being fired from his job at Lucent Technologies' Bell Laboratories in Murray Hill, New Jersey, he disappeared from public view.

In 2004, the physics department's doctoral committee at his alma mater, the University of Konstanz, asked Schön to return his doctoral certificate, citing a law that allows universities to rescind degrees when the recipient acts dishonorably, says university rector Ulrich Rüdiger. Schön filed an objection and last year sued the university. The judge in the case has now ruled that because Schön's misconduct wasn't related to his degree, the university can't take back his title.

"Scientific misconduct is a bit like doping in sports," Rüdiger says. "You can ban them from competition, but there are few legal penal-

They Said It

What am I in truth?
What am I in reality,
When only one in 10 of my cells
Is genetically humanity?

—From "Bacteria," one of the 20-odd songs celebrating the universe on scales from angstroms to astronomical units in "Powers of Ten," a choral work by composer David Haines. More than 200 singers from the Washington, D.C., area are set to perform the work on 10 October at the University of Maryland, College Park, kicking off the first inaugural USA Science & Engineering Festival. See www.usasciencefestival.org.

ties." He says university officials will receive the judge's full decision in October and then will decide whether to appeal. Schön is apparently working as an engineer for a company in Germany, Rüdiger says.

WHAT, US BITTER?

A nice piece of dark chocolate might sound tempting, but for many people in the Pamir Mountains in central Asia, the bittersweet candy would hardly be a treat.

That is one of the findings to emerge from an expedition by Italian scientists who followed in the footsteps of 13th century Venetian merchant Marco Polo along the ancient trading route known as the Silk Road. From July to September, they traveled more than 14,000 km from Trieste in Italy to Shanghai in China, hitting Georgia, Azerbaijan, Turkmenistan, Uzbekistan, Tajikistan, and Kazakhstan. Along the way, the team tested the reactions of more than 700 people to bitterness and saltiness, asked about their food preferences, took saliva samples, and tested their sense of smell and color and their sound perception.

The idea is to understand better how our genes—even those not directly tied to taste receptors—might influence what we like to eat, says Paolo Gasparini, a geneticist at the Burlo Garofolo Hospital in Trieste.

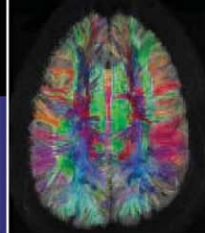
In the Pamir region, the team found that some 37% of people are "supertasters" extremely sensitive to bitter tastes, compared with 7% to 15% in most European countries. Indeed, a regional specialty is dried-apricot soup, which to a Westerner might sound more like dessert.





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MEDICINE
NOBEL
PRIZE
2010

ROBERT G. EDWARDS

NOBEL PRIZES

Honor for Test Tube Baby Pioneer

His work touched millions of lives and helped to create millions. Robert G. Edwards, the British physiologist who pioneered in vitro fertilization (IVF), was named the sole recipient of the 2010 Nobel Prize in physiology or medicine on 4 October. Edwards became a scientific celebrity after the birth of Louise Brown, the world's first "test tube baby," in 1978; since then, some 4 million children have been born following an IVF procedure.

Edwards now lives in a nursing home and is "very frail," says Martin Johnson, a professor of reproductive sciences at the University of Cambridge in the United Kingdom who was one of Edwards's first Ph.D. students.

He was unable to take the phone call from the Nobel Committee on Monday, and "realistically speaking" won't be able to pick up his medal in Stockholm in December, Johnson says. Michael Macnamee, chief executive of Bourn Hall, the fertility clinic that Edwards helped to found, said that Edwards was "very pleased indeed" when his wife, Ruth, told him the news. "He is aware of the award and of course is very happy to receive it," Macnamee says.

In the 1950s, Edwards was inspired by research demonstrating that rabbit egg cells

fertilized in the lab could give rise to offspring, and he set out to better understand the biology of human egg cells, sperm, and embryos. Working with slices of human ovaries given to him by a gynecologist, Molly Rose—who also delivered two of Robert and Ruth's five daughters—Edwards used trial and error to find the conditions that would coax human eggs to mature. With various colleagues he also pieced together how hormones regulate oocyte maturation, when the eggs can be fertilized by sperm, and the conditions necessary for sperm to activate and fertilize the egg. In 1969, he and his colleagues managed to fertilize a human egg in vitro for the first time, a feat that attracted massive media attention. But the resulting embryo didn't develop.

In 1968, Edwards struck up a fertile collaboration with gynecologist Patrick Steptoe, who—against skepticism among doctors and surgeons—had introduced the technique of laparoscopy, or "keyhole surgery," and used it to retrieve mature eggs from ovaries. "They were a perfect match. Both were pioneers, and they respected each other's skills and expertise," says Johnson. But the pair ran into strong social, religious, and medical opposition to their research.

Making babies. Edwards's research offered new hope to infertile couples.

"They were definitely swimming upstream," says embryologist and stem cell researcher Roger Pedersen of the University of Cambridge. In 1971, the U.K. Medical Research Council (MRC) turned down Edwards's request for funding—a decision that surprised and disappointed him.

One reason was that overpopulation was a growing concern at the time, says Johnson, who has become a historian of the field and who published a paper in July on the background of the MRC decision. "People said: Why would we help people having children when our main problem is how to prevent people from having children?" Other factors played a role as well, including worries that IVF might lead to abnormal fetuses. The fact that Edwards—not a physician himself—had become a media darling didn't help either.

Donations from private sources allowed Edwards and Steptoe to continue their work, but for years they failed to establish a successful pregnancy, in part because of the side effects of the hormone treatments given to patients to stimulate their ovaries to produce mature eggs. Eventually, they learned how to time their procedures to match their patients' natural menstrual cycles. That led to their 1978 triumph. After a second healthy baby had been born, MRC quickly reversed course and became an enthusiastic supporter of Edwards's work.

Steptoe died in 1988; because Nobel prizes are awarded to living scientists only, he could not be included in the prize. Had he been alive, he probably should have shared the honor, Johnson says. Edwards "was probably the more imaginative and creative of the two, but one shouldn't distract from [Steptoe's] remarkable and extraordinary contributions," he says.

Edwards's work made it possible for researchers to ultimately derive human embryonic stem cells, Pedersen notes, adding: "That was part of his original vision." But Karolinska Institute cell biologist and Nobel Assembly member Christer Höög—who authored the paper describing why Edwards deserved a Nobel—says that the prize was not intended to make a statement about stem cell research. "This award is only for the core technology" of IVF, he said.

CREDITS: BOURN HALL; © THE NOBEL FOUNDATION (INSET)



Although some still see IVF as controversial—the Vatican criticized the Nobel Committee’s pick this week—the treatment has become mainstream in most developed nations. But it’s still far from perfect. Only about 30% of attempts end in a live birth, despite the fact that doctors routinely place multiple embryos in patients to boost the chances of pregnancy. That’s in part because even in nature only 20%

of all embryos successfully implant in the womb—a fact that frustrated Edwards.

A prolific writer, Edwards was actively involved in the ethical debates around IVF from the start. As early as 1971, he published a paper in *Nature*, still regarded as a classic, that laid out the ethical, social, and regulatory issues in human embryology. He liked taking provocative positions, if only to flush out counterarguments, Johnson

says. He disagreed with the scientific community’s decision to declare human cloning off-limits without discussing the potential benefits. “I’ve never met anyone worth cloning, including myself,” Edwards once quipped. “But to him, closing the debate was the antithesis of scientific inquiry,” says Johnson.

—GRETCHEN VOGEL
AND MARTIN ENSERINK

NOBEL PRIZES

Still in Its Infancy, Two-Dimensional Crystal Claims Prize

This year’s recipients of the Nobel Prize in physics earned that honor with the most wafer-thin of discoveries and with the help of some Scotch tape. Andre Geim and Konstantin Novoselov of the University of Manchester in the United Kingdom share the prize for their discovery in 2004 of graphene, a one-atom-thick material made of carbon. Only a few years later, the material promises revolutionary advances in electronics and other technologies. “My hope is that graphene ... will change our everyday lives the way plastics did,” Geim says.

Graphene had humble beginnings. Geim and Novoselov first produced flakes of it by peeling them off a chunk of graphite using cellophane tape. “It just started as a Friday evening experiment,” Novoselov recalls. “We were enjoying doing it.” Others had been trying more complicated methods of liberating a single sheet of atoms, says Philip Kim, a physicist at Columbia University. “I was shocked that they were able to get a single layer with such a simple method,” Kim says. “We were completely scooped.”

Early on, graphene fascinated primarily theoretical physicists. Because of the material’s two-dimensional nature, the electrons in it conspire to move as though they have no mass, much like particles of light. So just like photons, the electrons must always move, cruising along at their own specific “speed of light.” All of this was predicted decades ago, but graphene provided the first

example of such odd behavior.

Quickly, physicists, engineers, and chemists began to see the potential for applications. Graphene has many bizarre and often contradictory properties. For example, it is flexible like plastic but stronger than diamond, and it conducts electricity like a metal but is transparent like glass. Researchers have figured out how to make sheets measuring tens of centimeters across. They have

been so much progress in its properties and mass production,” he says.

In that regard, this year’s prize could be considered an anomaly. In the past, a few prizes have quickly spotlighted discoveries that upended the prevailing theory; others have recognized advances that over decades had led to ubiquitous applications. This year’s prize, by contrast, honors physics that by all accounts is beautiful but not revolutionary.

“You don’t need a new theory” to understand graphene, says Jeroen van den Brink, a theorist at the Institute for Materials Sciences at the Dresden University of Technology in Germany. At the same time, it celebrates the potential for applications yet to come. “Will this really come into the market?” Kim says. “I think it’s really difficult to say.” Still, everyone interviewed by *Science* says Geim and Novoselov thor-

oughly deserve the prize.

The prize is also unusual because one recipient has another claim to fame. In 2000, Geim won a share of an Ig Nobel Prize, a satirical award given out by the publishers of the *Annals of Improbable Research*, for magnetically levitating a live frog. Geim is the first person to win both prizes. During a press conference announcing the Nobel Prize, Geim reacted with unusual candor to his latest award: “When I got the telephone call, I thought, ‘Shit!’ because it is a life-changing event.”

—ADRIAN CHO

With reporting by Daniel Clery.



ANDRE GEIM



PHYSICS
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PRIZE
2010



KONSTANTIN
NOVOSELOV

developed methods to cut the sheets into nanometer-scale patterns and to change graphene’s electrical properties by, for example, affixing hydrogen to its surface.

That’s opened the way to scads of possible applications. The South Korean electronics company Samsung has developed a touch screen from graphene, whereas researchers with IBM have fashioned ultrafast transistors from the stuff. A sieve of graphene might also serve to sequence DNA. Even Novoselov says it’s too early to say exactly what uses graphene will find. “Whatever I say now will be wrong because there has

Hidden history. Susan Reverby found records describing unethical studies.



BIOMEDICAL ETHICS

Guatemala Study From 1940s Reflects A 'Dark Chapter' in Medicine

After making a public apology, the United States this week is launching two inquiries into a stunningly unethical U.S. medical study that was conducted in Guatemala 64 years ago. The research—funded from 1946 to 1948 by the National Institutes of Health (NIH)—infected hundreds of Guatemalan prisoners, soldiers, and mental health patients with syphilis and other sexually transmitted diseases. Its aim was to track the course of infection and test new preventive treatments. But the doctors who ran it for the U.S. Public Health Service (PHS) never published results.

The work was “clearly unethical,” said Secretary of State Hillary Clinton and Health and Human Services Secretary Kathleen Sebelius in a joint statement of regret on 1 October: “We are outraged that such reprehensible research could have occurred under the guise of public health.” In a telephone press conference, NIH Director Francis Collins also deplored this “appalling example” from “a dark chapter in the history of medicine.” NIH plans to fund a “fact-finding investigation” into what happened in Guatemala, run by the National Academies’ Institute of Medicine. At the same time, the U.S. Presidential Commission for the Study of Bioethical Issues is putting together a group of international experts, Collins said, “to ensure that all human medical research conducted around the globe today meets rigorous ethical standards.”

The Guatemala project came to light after Susan Reverby, a medical historian at Wellesley College in Massachusetts, found a batch

of forgotten records donated to the University of Pittsburgh by John C. Cutler, an expert in sexually transmitted diseases. Cutler died in 2003. Reverby was looking into another study she has written about—the 1932–1972 Tuskegee experiment in Alabama, in which researchers left African-American men with syphilis untreated in order to observe the disease. Cutler was involved in both studies.

With the approval of his PHS bosses, Cutler set up a program at the U.S. Venereal Disease Research Laboratory in Guatemala City to recruit prostitutes (legal in Guatemala) and use them to spread disease among uninfected men. Guatemalan officials approved the plan, allowing researchers and disease-spiked prostitutes into a prison and an army barracks. They also used direct inoculation with infectious material, particularly for patients in a mental health asylum. (Children in an orphanage were involved, too, but only as blood donors.) None gave consent, and it’s likely that participants were deceived about the nature of the experiments.

Cutler’s team had at least two goals, Reverby writes in a report to be published in a January 2011 edition of the *Journal of Policy History*. One was to test reactions to “fresh infective material,” apparently in hopes of finding a way to enhance immune responses. And the second was to test chemoprevention methods to be used after exposure to bacteria. Specifically, Reverby says, they wanted to come up with a penicillin-based formula that would be better than the “pro kit” given to U.S. soldiers at the time—a concoction of

calomel and mercury that was supposed to be rubbed on the penis after unprotected sex. “As you can imagine, nobody was using it because it burned,” says Reverby. The doctors “didn’t know what penicillin could do” and thought it might work better.

Partway through, the study apparently began to implode. Cost was a problem. The doctors treated subjects with penicillin when an infection was confirmed; this required more than 3 million units of the valuable drug. Meanwhile, even Cutler was having trouble with the deception. Reverby quotes a letter from Cutler to a colleague in 1947 in which he writes: “As you can imagine, we are holding our breaths, and we are explaining to the patients and others concerned ... that the treatment is a new one utilizing serum followed by penicillin. This double talk keeps me hopping. ...” The project quietly faded away in 1948.

Reverby presented her investigation at a meeting of medical historians in May. She says there were about 20 people on hand for her talk on a Sunday morning: “Needless to say, it got no press coverage.” Later, she forwarded a draft to the former director of the Centers for Disease Control and Prevention (CDC), David Sencer, who “sent it up the chain of command.”

In July, CDC looked at the files and essentially validated Reverby’s report. CDC’s summary, released on 1 October, says data suggest that 696 subjects were exposed to syphilis, 772 to gonorrhea, and 142 to chancroid. The corresponding infection rates were roughly 61%, 30%, and 97%. As far as is known, most were treated, but records suggest that only 76% of those directly inoculated and infected with syphilis received “adequate” amounts of penicillin.

Monetary compensation should be considered for the victims or their descendants, according to Johns Hopkins University bioethicist Ruth Faden—although fixing responsibility would not be simple. And although rules are in place to prevent something like this from happening today, Faden says, “we’re reminded again of what can happen if we drop our guard.”

Reverby says these revelations are likely to revive rumors that men in the Tuskegee study were deliberately infected. After sifting Tuskegee’s records for 20 years, she is “absolutely positive without a shadow of a doubt” that this did not happen. And why did the doctors in Guatemala go over the edge? “I think they just really wanted their results; they fell in love with their data.” If so, it was a blind love.

—KRISTEN MINOGUE AND ELIOT MARSHALL

CREDIT: COURTESY OF WELLESLEY COLLEGE

NEWSMAKER INTERVIEW: KIM HEUNG-KWANG AND PARK CHAN-MO

Will Korea's Computer-Savvy Crown Prince Embrace Reform?

SEOUL—It's a rare day that North Korea watchers successfully predict momentous changes in the hermit kingdom. But last week, the heir apparent to top leader Kim Jong Il emerged from the shadows—and as anticipated, it is his third son, Kim Jong Un.

Intelligence sources and organizations with informants in the North have been sharing what they have learned about the mysterious young Kim, who is thought to be 27 years old. According to internal North Korean propaganda, informants claim, Kim oversees a cyberwarfare unit that launched a sophisticated denial-of-service attack on South Korean and U.S. government Web sites in July 2009. South Korea's National Intelligence Service blamed the North, which has not commented publicly on the attack. Kim Jong Un's involvement cannot be confirmed, says computer scientist Kim Heung-Kwang, founder of North Korea Intellectuals Solidarity, a group of university-educated defectors that raises awareness of conditions in the North and first revealed North Korea's botched currency reform late last year. But it's plausible: Kim claims that Kim Jong Un was tutored privately by a "brilliant" graduate of Université Paris X who chaired the computer science department at Kim Chaek University of Technology in Pyongyang before disappearing from public view in the early 1980s.

Analysts can only speculate whether Kim Jong Un's presumed familiarity with cyberspace and his exposure to the outside world—he attended private school in Switzerland—will influence North Korean policy anytime soon, if at all. Science diplomacy buffs have their antennae up for signals on how last week's developments might influence international collaborations, including a new tuberculosis laboratory (*Science*, 12 March, p. 1312) and Pyongyang University of Science and Technology (PUST) (*Science*, 25 September 2009, p. 1610).

For insights into how things may shake out for North Korea's scientific community, *Science* spoke with Kim Heung-Kwang and Park Chan-Mo. Kim Heung-Kwang, 50, is one of five Ph.D.-trained defectors known to reside in South Korea. (Defectors num-

ber 20,000 or more; only a few dozen hold master's or higher degrees.) He grew up in Hamhung, North Korea's second largest city, and studied at Kim Chaek University of Technology before working as a professor at Hamhung Computer College and Hamhung Communist College. Embarking on what he calls "a big adventure," Kim Heung-Kwang crossed the Tumen River into China in September 2003. "I wanted to work with computers in a free environment," he says. A year later, he paid a broker to smuggle his wife and daughter to the South. His organization is now raising funds to launch a think tank, the North Korea Development Institute, in 2011.

A specialist on computer graphics and simulation, Park Chan-Mo, 75, last week stepped down as the first president of the National Research Foundation of Korea to

put pressure on his own people at first. The leaders think their people should starve a little bit to make them calm. But Kim Jong Un is a young person with a background in information technology, so he may desire to transform North Korea from a labor-intense economy to a knowledge economy like South Korea is doing. From the outside, there may not appear to be much of a difference, but inside the government this Generation X'er may start to make positive changes.

Q: Some South Koreans worry that the North will use PUST to acquire knowledge for its weapons programs. Is it a risky venture?

K.H.-K.: If North Korea keeps its promises [to permit academic freedom and Internet access], it will be a success.

P.C.-M.: Our purpose is the globalization of North Korea through PUST. In that way, their economy can gradually develop, which will make it easier for reunification later.

Q: Is it an auspicious time for the West to reach out to North Korea's scientific community?

K.H.-K.: It's very important to do this, especially with the severe tensions right now between North and South. If Western scientists sincerely try to engage North Korea, there will be a positive response. North Korea has solid expertise in computer algorithms and software development. Collaborations in these areas can be

win-win for both sides.

Q: How do you reassure the North Korean government that scientific cooperation isn't intended to undermine the regime?

P.C.-M.: We never say anything bad. But we have to tell them there are certain things that need to change. For example, one day at an institute in Pyongyang we heard a siren. We thought there was a fire. Our hosts said don't worry, it just means the electricity will be shut off in half an hour. They badly needed a stable electricity supply for their research, so we suggested that they install UPS [uninterruptible power supply] equipment.

Our first aim is to build trust, to show that we are not brainwashing them. Wouldn't it be great if someday [smiling] a collaboration with North Koreans will publish a paper in *Science*?



Reaching out. Kim Heung-Kwang (left) and Park Chan-Mo advocate engaging North Korea.

devote his time to PUST, which he is helping establish. Foreign faculty members will begin teaching PUST's inaugural class—100 undergraduates and 60 grad students—later this month. In a remarkable gesture for a nation that permits few of its citizens e-mail or Web access, North Korea has promised PUST a campuswide Internet connection; this is not yet operational. —RICHARD STONE

Q: Kim Jong Un will need army backing to consolidate his power, so he presumably will maintain North Korea's Songun [Military First] policy. But will his experience abroad have made him a more open-minded person?

K.H.-K.: Anyone who comes to power will

CELLULAR REPROGRAMMING

New Technique RiPS Opens Stem Cell Field

While stem cell scientists in the United States are grappling with continued uncertainty about the future of federal funding for work with embryonic cells (see p. 163), the field got a bit of good news last week: a paper describing a new method for prompting mature cells to take on a different fate. The technique is cleaner, safer, faster, and more efficient than recently developed methods for reprogramming adult cells, promising to give researchers a powerful new tool for making and using stem cells. It “will have a huge impact in the near term,” says Justin Ichida, who studies reprogramming at the Harvard Stem Cell Institute and was not involved in the study.

Four years ago, scientists took a major step toward overcoming the biggest ethical hurdle in stem cell research. Instead of using cells derived from embryos, researchers found a way to make adult cells behave as though they were embryonic. Simply inserting extra copies of four genes into these cells gave the cells the ability to develop into almost any cell type in the body. Known as induced pluripotent stem (iPS) cells, these cells are a potential boon for studying and ultimately treating a variety of diseases, and many labs immediately added the technique to their repertoire. Scientists have also used the trick to turn one mature cell type directly into another (skipping the embryonic stage) by inserting key genes for the desired cell type.

But the technique, called cellular reprogramming, has some drawbacks. The reprogrammed cells retain copies of the inserted genes, which makes them prone to forming tumors and could potentially skew experimental results. And there is some evidence that iPS cells aren’t exactly like embryonic stem cells in their gene expression, retaining a subtle cellular memory of the tissue they came from. The method is also relatively inefficient, reprogramming only about one out of 1000 cells exposed to the treatment, and it takes more than a month for iPS cells to appear.

The new technique goes a long way toward fixing those problems. Stem cell researcher

Derrick Rossi of Harvard Medical School in Boston and his colleagues used synthetic RNA molecules that correspond to the genes inserted in classic reprogramming techniques. The technique makes iPS cells in about half the time, they reported online last week in *Cell Stem Cell*. And because the RNA quickly breaks down, the reprogrammed cells are genetically identical to the source cells.

Rossi says his first attempts to use RNA to induce protein production were stymied by cells’ innate antiviral defenses, which attack foreign RNA and can trigger programmed

Other researchers have been racing to find other methods to reprogram cells, but most of them have proved less efficient than the classic technique.

Further experiments suggest that the RNA approach does a more thorough job of reprogramming the cell than other methods. The genes that RiPS cells express are very similar to those expressed by ES cells—in other words, they seem to be a closer match to ES cells than most iPS cells to date. The method can also prompt cells to become nonembryonic cell types. By inserting synthetic RNA that codes for a key gene in muscle tissue, for example, the researchers could turn both fibroblasts and RiPS cells into muscle cells.

“I’m so impressed . . . that we are going to turn over our entire iPS core to this new method to make stem cells from patients with all sorts of diseases,” says stem cell researcher Douglas Melton of Harvard’s Stem Cell Institute. “It is a major advance.”

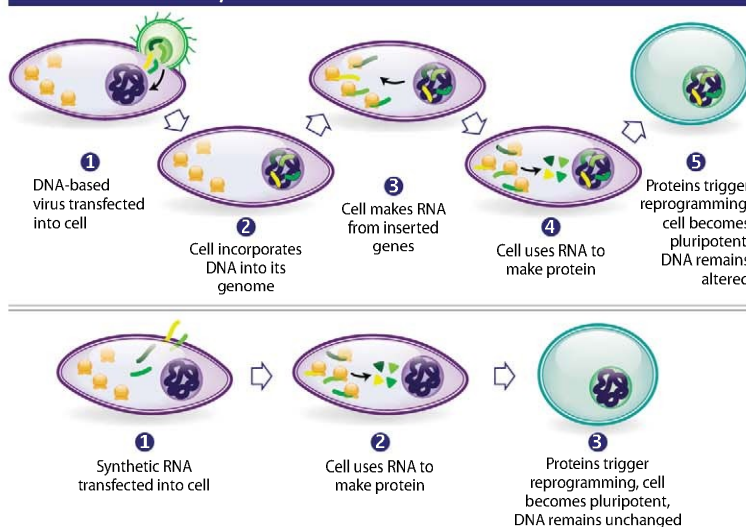
Rossi says the synthetic RNAs will be easy for other labs to make: “If you have basic molecular biology tools, you can make these RNAs.” Still, Ichida says he is not giving up his search for a suite of chemicals that can reprogram cells. For researchers who might want to make thousands of iPS cell lines, he says, RNA reprogramming will still be time-consuming and expensive, and a chemical cocktail could prove even more efficient, he says. Others agree. Although he says the RNA technique is a significant advance, stem cell researcher

James Thomson of the University of Wisconsin, Madison, says it is too early to discount other approaches to reprogramming.

The RNA technique could have uses beyond the stem cell field, Rossi and others note. The modified RNA can also prompt cells to make designer proteins, Rossi says. And developmental biologists can use it to better understand the effects of certain genes. “It’s the flip side of RNAi,” Rossi says, referring to a widely used technique in which scientists use RNA to block the expression of genes in cells. Rossi says he will be exploring how to use the RNA-prompted protein expression to replace proteins in diseased patients. “If we could reprogram somatic human fibroblasts to pluripotency, you can do anything with this technology,” he says.

—GRETCHEN VOGEL

REPROGRAMMING, OLD AND NEW



Cleaner, safer, faster. A new technique using synthetic RNA can reprogram adult cells so they are genetically identical to the source cell.

cell death. But he and his colleagues discovered that by substituting slightly modified versions for two of RNA’s usual bases, they could make synthetic RNAs that the cell accepted as its own. By inhibiting interferon—a key part of the cells’ anti-RNA defense—they got the cells to express even more of the desired proteins. When the researchers applied a daily cocktail of these synthetic RNAs to connective tissue cells called fibroblasts, the cells dedifferentiated into embryonic-like cells. The team calls its cells RiPS cells, for RNA-induced pluripotent stem cells.

To the team’s surprise, the process took just over 2 weeks and reprogrammed as many as 4% of the cells in the culture dish. That makes it roughly 100 times more efficient than the gene-transfer technique and twice as fast.



In limbo. The courts will soon decide whether U.S.-funded research on human embryonic stem cells is legal.

COURT BATTLE

What's Next for Stem Cell Research?

Last week's appeals court decision to temporarily lift a ban on the use of federal funds for research with human embryonic stem cells (hESCs) was good news for researchers. But the legal battle is far from over. A decision on the underlying court case could come by late November. If the courts agree that hESC research violates a ban on taxpayer-funded research that harms embryos, hESC work could be shut down again. And the prospects that Congress will come to the rescue remain unclear. Given the uncertainty, some researchers say they are removing hESC work from their National Institutes of Health (NIH) grant applications.

The saga began on 23 August, when U.S. District Court Chief Judge Royce Lamberth issued a preliminary injunction halting federal funding for hESC research while he considered a lawsuit brought by two researchers who study adult stem cells (*Science*, 17 September, p. 1450). The suit contends that the NIH guidelines under which it is funding hESC research are illegal. For 17 days, NIH suspended grant reviews and payments and eventually shut down intramural research on hESCs. Then on 9 September, the U.S. Court of Appeals for the D.C. Circuit suspended, or "stayed," the injunction. On 28 September, a three-judge panel of the appeals court replaced the brief stay with a longer one until it hears the government's appeal of the injunction.

During the two stays, NIH issued award letters for 24 grants for which annual payments were due in September. Grant reviews also seem to be back on track, according to hESC researchers.

Exactly what comes next in the courts is unclear. Both sides have asked Lamberth to decide the underlying case without a trial; they've agreed on a final deadline of 28 October to submit documents. Lamberth

must determine whether NIH's July 2009 stem cell guidelines violate the Dickey-Wicker law banning federal funds for research that harms embryos, and whether NIH followed the correct procedures in developing the guidelines.

The appeals court is also moving ahead with the appeal of Lamberth's preliminary injunction. The last deadline for briefs is 4 November, then the three-judge panel will hear oral arguments. If, as expected, Lamberth rules in favor of the plaintiffs on the full case, the government will appeal and the case could be consolidated with the appeal of the preliminary injunction. (Some observers expect the stay would remain in place.) Another possibility is that Lamberth will wait to see how the appeals court rules on the preliminary injunction before he decides the case itself. Whichever court goes first, a decision could come by late November.

If the appeals court rules against NIH, in-house research and hESC reviews and grants will be frozen again. The research community has geared up to blitz lawmakers with letters urging them to pass a law legalizing the NIH guidelines, says Anthony Mazzaschi of the Association of American Medical Colleges. But Congress will face a logjam of bills when it returns in November for a brief session.

Michael Kyba of the University of Minnesota, Twin Cities, is one who is "extricating" hESCs from his applications and will instead use induced pluripotent stem cells, which are created from adult cells and don't carry the same ethical baggage. He will use private funds for any essential hESC work. "The legal situation is clearly driving the research project planning regarding use of hESCs rather than scientific rationale," says stem cell researcher Timothy Kamp of the University of Wisconsin, Madison.

—JOCELYN KAISER

Science Insider

From the Science Policy Blog



A group of states bordering the Gulf of Mexico will dole out \$500 million for research on the impacts of the BP oil spill. To be spent over 10 years, the Gulf of Mexico Research Initiative has already distributed a total of \$30 million to five consortia of gulf universities and \$10 million to the National Institutes of Health. http://scim.ag/gulf_money

A new report from the U.S. National Academies says the fastest way to train more **minority scientists and engineers** is to improve the retention and completion rates of college students already interested in the natural sciences and engineering. That approach, it says, will also boost the overall number of students going into scientific fields. http://scim.ag/minority_science

Howard Hughes Medical Institute (HHMI) has embraced **plant science** by allocating \$75 million for a new 5-year program that will pay the salaries of up to 15 plant biologists. "Plants are as good a model system as any other for studying biological processes," says HHMI President Robert Tjian in announcing the new batch of HHMI investigators. http://scim.ag/hhmi_plants

A policy to **manage gray wolves** via recreational hunting relies on faulty ecological science, say two ecologists. Their research challenges a long-held assumption that wolf populations won't be decimated by hunting. Ecologists had believed that hunters could remove 28% to 50% of the animals in a population without causing long-term harm to their numbers. http://scim.ag/wolf_hunt

A report requested by the White House Office of Science and Technology Policy says the federal government needs an organized effort to develop **effective strategies to adapt to a changing climate**. These approaches should include regional partnerships between scientists and local planners and resource managers as well as educational programs to train new experts in adaptation. Sharing data is another priority. But the efforts don't require a lot of new money, the report said. http://scim.ag/adapt_report

For more science policy news, visit <http://news.sciencemag.org/scienceinsider>.

BRAIN RESEARCH

Neuroscientists Grapple With Their Field's Big Questions

SEATTLE—A shrine to rock music is an unusual place to hold a scientific conference, but last week the Experience Music Project museum here hosted 18 leading neuroscientists who gathered to discuss the major open questions in their field and the as-yet-to-be-invented technological advances that could best help solve them. The benefactor of both the museum and the conference was Microsoft co-founder Paul Allen, who has so far poured \$126 million into brain research via the Allen Institute for Brain Science, launched in 2003.

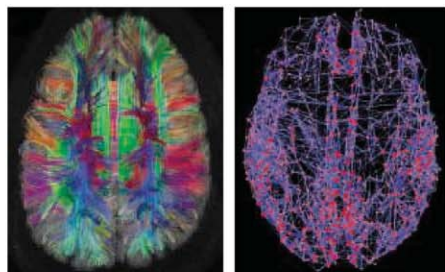
The Allen Institute has specialized in using brute-force technology—laboratory robots, automated microscopes, and powerful computers—to create atlases of gene activity throughout the brain (*Science*, 29 September 2006, p. 1879). The fruits of this effort are freely available to researchers. In a short chat with journalists, Allen said the institute was looking for ideas for what to undertake next. “There’s no shortage of amazingly challenging problems,” he said. “What we try to bring to it is a unique high-throughput industrial-scale approach.”

Spurning PowerPoint slides and speaking extemporaneously, Sydney Brenner, a 2002 Nobel laureate, posed questions about how the complexity of the nervous system is encoded in the genome. Some researchers pondered the complexity of synapses and the role of glial cells, which outnumber neurons in the human brain. Others described their efforts to understand the neural circuits underlying memory, perception, and decision-making.

Several scientists presented a wish list of future technologies to help solve the questions they’d posed. “A field is often brought forward as much by new technology as by new science and new ideas,” said Michael Dickinson of the California Institute of Technology in Pasadena. Nobel laureate Susumu Tonegawa of the Massachusetts Institute of Technology (MIT) in Cambridge wished for a noninvasive method

to simultaneously record the activity of many individual neurons in the human brain in real time. “I have great confidence that something like that will happen, and it will revolutionize our understanding of the human brain,” Tonegawa said, adding that he didn’t expect to see it in his lifetime.

Other speakers described promising work already under way to help bridge the gaps between genes, cells, circuits, and behavior. Edward Boyden of MIT talked about his lab’s efforts to develop new optogenetic tools, which have taken neuroscience by storm in recent years (*Science*, 15 December 2006, p. 1674). Using genetic tricks, scientists can insert light-sensitive proteins into specific populations of neurons and then use pulses of laser light to perturb their firing. Boyden



Making connections. Array tomography (top) provides a close-up view of connections between neurons, whereas diffusion spectrum imaging (left) helps map connections among brain regions (right).

has been expanding the optogenetics tool kit by combing fungi, archaeobacteria, and other organisms for additional light-sensitive proteins that can be used to stimulate or inhibit neurons on different time scales or across smaller or larger areas of the brain. He’s also been developing optical fibers that can deliver light at several locations. Arrays of such fibers implanted in mouse brains could one day let researchers manipulate multiple components of a neural circuit in freely behaving animals.

Stephen Smith of Stanford University in Palo Alto, California, wowed the audience with a video tour of a 3D reconstruction of mouse cerebral cortex created by “array tomography,” a method his lab has developed. Researchers first cut ultrathin sections of brain tissue, each of which can be stained multiple times with fluorescent markers for proteins of interest. Microscopes capture high-resolution images of each section, and a computer stitches them together. The result is a brightly colored forest of axons and dendrites dotted with synapses. Smith wants to use the method to examine how synapses differ in structure and function. “Synaptic diversity is a formidable foe that undermines everything we’re trying to do in terms of understanding the brain,” he said. “We don’t understand it.” The video, accompanied by a soundtrack composed by Smith’s daughter, will be included in the supplementary online material for a paper in the 18 November issue of *Neuron*. The caption includes instructions to play it on “good speakers turned up loud,” Smith said. “I think it’s the first time that phrase has appeared in *Neuron*.”

Panning out from the connections between neurons to the connections between regions of the human brain, Olaf Sporns of Indiana University, Bloomington, argued for a more quantitative approach to analyzing networks of brain regions. Sporns compared today’s understanding of connectivity in the human brain to 15th century geography: Some areas are mapped much better than others. That may change with the new Human Connectome Project, funded by the U.S. National Institutes of Health, which announced its first round of grants in mid-September. The project will map the brains of 1200 volunteers using several magnetic resonance imaging methods, including a relatively new technique called diffusion spectrum imaging, which provides detailed images of the axon tracts that carry signals between brain regions. The inclusion of many twins and family members in the study should help researchers look for genetic influences on neural wiring, Sporns said. The project should also mesh with the Allen Human Brain Atlas of gene expression launched earlier this year, enabling researchers to investigate whether brain regions that are physically connected express similar genes and participate in similar functions.

Allan Jones, the chief executive officer of the Allen Institute, said it’s too early to speculate about specific projects the institute might tackle as a result of the meeting, but there won’t be a lack of ideas, he said: “There’s so much to do.”

—GREG MILLER

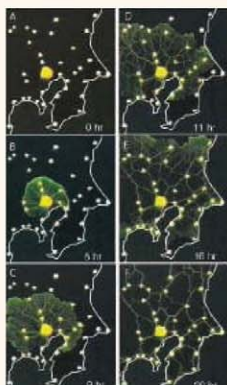
CREDITS (TOP TO BOTTOM): K. MICHEVA, B. BUSSE, N. WELER, N. O’ROURKE, S. J. SMITH, STANFORD UNIV.; P. HAGMANN/EPFL, CHUV, LAUSANNE, CH.; V. WEDEEN/MGH, AA MARTINOS CENTER/HUMAN CONNECTOME PROJECT (LEFT); P. HAGMANN ET AL., PLOS BIOL. 6, 7 (JULY 1, 2008)

From *Science's* Online Daily News Site

Another Earth?

The few rocky extrasolar worlds found so far are either too close or too far from their stars to fall within the so-called habitable zone, in which liquid water—and therefore life—could exist. But discoverers of a new Earth-like planet called Gliese 581g say it could be a game-changer.

Detected from the minuscule amount of gravitational influence it exerts on its star, Gliese 581g lives a mere 20 light-years away in the constellation Libra. Although its orbit brings it closer to its parent star than Mercury is to our sun, the star shines with only about 1% of the sun's brightness. That means the planet could harbor life, the researchers report in a paper to appear in *The Astrophysical Journal*. <http://scim.ag/other-earth>



Ig Nobel Prizes

Researchers who demonstrated that swearing alleviates pain and a team that found fellatio in fruit bats were among the winners of this year's Ig Nobel prizes, given out at a ceremony last week at Harvard University. The prizes recognize studies that "first make people laugh, and then make them think," according to the Ig Nobel committee. Also among the laureates: a group that recreated Tokyo's transportation network by growing a slime mold. <http://scim.ag/Ig-Nobels>

Hot Spot

Researchers have uncovered the largest geothermal hot spot in the eastern United States.

With the help of Google.org, the philanthropic arm of the search-engine giant, scientists used rock temperatures collected by companies drilling for oil and natural gas to create a geother-

mal map of the United States. The map revealed an 18,700-square-kilometer area in West Virginia where temperatures soared as high as 200°C at depths as shallow as 5 kilometers. That's comparable in depth to geothermal hot spots currently being tapped in Nevada and California. <http://scim.ag/hot-spot>

Read the full postings, comments, and more at <http://news.sciencemag.org/sciencenow>.

SCIENTIFIC EXCHANGES

A Chill in China-Japan Academic Relations

TOKYO—A diplomatic tiff stemming from a collision last month between a Chinese trawler and two Japanese coast guard vessels has chilled academic exchange between the two countries. Top Chinese scientists have canceled appearances at meetings in Japan in recent days, and negotiations between two universities over educational exchange are frozen. Japanese scientists say they have not received an official explanation from Chinese counterparts, and no one knows how long the chill will last.

The first sign that the incident would affect scientific ties came when a delegation of 15 or so researchers headed by Chinese Academy of Sciences President Lu Yongxiang pulled out of a bilateral meeting on sustainability scheduled for 29 September. "There was no explanation; we just suddenly got a fax canceling their attendance," says engineer Hiroyuki Yoshikawa, former president of the University of Tokyo and one of the meeting organizers. It was to be the fourth in a series of annual meetings held alternately in China and Japan that bring

together top researchers from the two countries. This year's discussions were to focus on low-carbon technologies.

Also affected was the annual Science and Technology in Society Forum held 3 to 5 October in Kyoto. Several Chinese delegates canceled at the last minute, including Chen Zhu, China's health minister and a leading cancer researcher. And *Science* has learned that an official of a major Japanese university went to China last week to sign a cooperation agreement with a Chinese university, but his counterparts were suddenly unavailable. The Japanese official asked that the universities not be identified to avoid harming delicate negotiations to resolve the impasse.

The 7 September collision that apparently prompted the academic chill occurred near what Japan calls the Senkaku Islands, about 185 kilometers northeast of Taiwan and 170 kilometers north of Ishigaki Island, part of Japan's Okinawa Prefecture. Japan has controlled the uninhabited islands since the late 1800s, but Taiwan and China claim

them as part of Taiwan and call them the Diaoyu Islands. After the collision, Japan held the captain for possible prosecution under domestic law, infuriating China. After an escalating war of words, Japan released the captain on 24 September. China has demanded that Japan apologize and pay compensation; Japan has demanded compensation for damage to its boats.

To underline its displeasure, China has canceled a number of governmental visits in other spheres and withdrew permission for a 1000-strong Japanese youth group to visit the Shanghai Expo. Chinese customs officials also reportedly blocked exports of rare earth minerals to Japanese firms, although China denied cutting off supplies.

Scientists on both sides are hoping for a quick return to normality. "S&T collaboration between the scientific communities from the two countries should be promoted," says a top Chinese government scientist. "We're scientists," says Yoshikawa. "We don't want these tensions to affect cooperation."

—DENNIS NORMILE



Grave Disputes

As U.S. legislation requiring the return of Native American remains to tribes turns 20, a new controversy threatens the tenuous relations between the scientific and Native American communities



"NO DEBATE; REPATRIATE!" WAS THE CHANT of protesters standing outside the chancellor's home at the University of California, San Diego (UCSD), on a winter's day last year. The focus of their ire: 10,000-year-old Paleoindian bones found in 1976 during excavations at the former chancellor's home. The local Kumeyaay Nation wanted to remove the remains from a university collection and return what they believe are their ancestors to Mother Earth.

More quietly, but just as passionately, the university's anthropologists argued in nearby conference rooms that the rare ancient bones have no direct relation to the tribe and should be kept for scientific analysis. The remains, they said, could help illuminate the still-mysterious question of how and when humans migrated from the Old World to the New. Today, the sought-after bones remain

◀ No debate. Native American protesters demand the return of UCSD's bones.

locked away in a neutral facility.

Twenty years ago, Congress passed a law aimed at laying to rest such arguments between scientists and Native Americans, and government, university, and Indian representatives will gather in Washington, D.C., on 15 November to commemorate the anniversary. But the debate over the Native American Graves Protection and Repatriation Act (NAGPRA), which gives Indians a chance to reclaim their ancient dead, is very much alive. The Department of the Interior office that oversees NAGPRA came under fire this summer from the U.S. Government

Accountability Office (GAO) for poor record keeping, questionable decision making, and inadequate resources. And new rules put into effect in May extend the law to give tribes like the Kumeyaay a way to recover even those ancient bones that cannot be linked to an existing people.

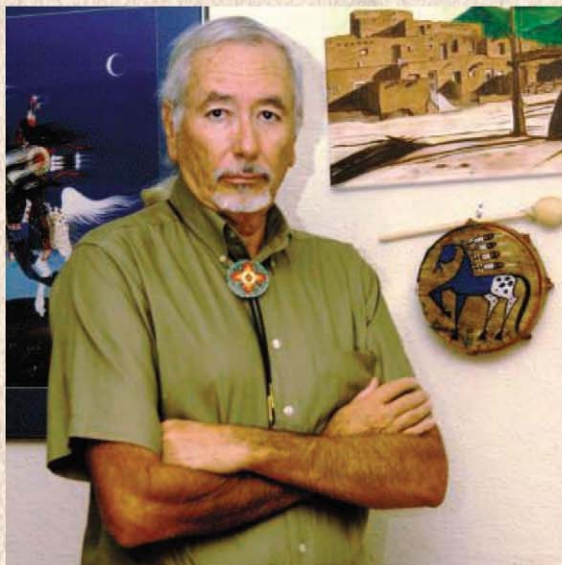
Neither Kumeyaay nor UCSD officials will say if the new regulations tilt the battle in favor of the tribe. But the controversy over the revised law exposes the divide between some Native Americans and scientists. "Anyone deceased should be allowed a decent burial," says James Riding In, an American Indian studies professor at Arizona State University (ASU), Tempe. "These are not just specimens for study." Yet scientific researchers say valuable data on past North American societies may be

Online

sciencemag.org

Special features include an expanded interview with archaeologist Stephen Lekson and a podcast interview with editor Elizabeth Culotta.

CREDITS (TOP TO BOTTOM): JOHN WARNER; ADRIANE TILLMAN/TA; JOLLA VILLAGE NEWS



Rescue mission. ASU's James Riding In says remains must be reburied.

at all, in part because of its great age (*Science*, 30 July 2004, p. 591). And in other cases, Native Americans felt that institutions were using the law's "unaffiliated" category to block repatriation. So they pushed for changes that would give them an opportunity to recover those remains as well. In May, the Department of the Interior implemented rules that allow tribes to request "culturally unaffiliated" remains found on their current or historical lands. The new rules affect roughly 120,000 Native American and Hawaiian remains.

Many Native Americans complain that the rule doesn't go far enough, because it exempts unaffiliated ceremonial objects and grave goods. And some researchers predict that the impact on science will be limited because Indians simply won't ask for large numbers of bones. Some groups no longer remember traditional ceremonies, have taboos on handling the dead, or lack the necessary time, money, and organization. "Tribes don't have the facilities or the personnel to handle this stuff," says Wilcox. "Much of it will remain in the collections."

objects and repatriate them to "culturally affiliated" tribes. Some items were exempt, including objects and remains that could not be linked to a particular tribe and those found on private land.

Repatriations proceeded, though slowly in many cases. By the end of 2009, federal agencies had reported giving back about 9000 or 55% of "affiliated" human remains and 130,000 or about 68% of associated funerary objects, according to a recent GAO report.

However, in the most famous repatriation case, scientists won a lawsuit arguing for the right to study a 9400-year-old Paleoindian dubbed Kennewick Man, which they said could not be affiliated to Native Americans

But others foresee disaster. The new rules are "draconian," says archaeologist Stephen Lekson of the University of Colorado Museum of Natural History in Boulder, and make it much easier for tribes to request unaffiliated objects. After the original law passed, says Kintigh, "I was not one of those who said the sky would fall." But he fears the new rule will deny researchers access to crucial specimens forever. The American Association of Physical Anthropologists argued in a 10 May letter to Hutt that the rule "could effectively remove ... human remains that document the rich and complex biocultural history of the first Americans." The result, it warned, could be "wholesale reburial of indigenous history." The Society for American Archaeology took a softer line, criticizing the rule for failing "to recognize scientific study as an important part of increasing knowledge about the human past."

Counting bones

After 20 years, just how much has NAGPRA affected research? Individual opinions vary, and answers based on quantitative analyses are hard to come by. But several researchers have tried. Physical anthropologist Elizabeth Weiss of San José State University in California examined osteological graduate work from

Walking in Two Worlds

Archaeologist and Choctaw Indian Dorothy Lippert gives Native American remains and sacred objects back to tribes as part of her job at the Smithsonian Institution in Washington, D.C. But sometimes her work becomes personal. Lippert, 43, recalls that in 2003 she learned that the museum held the skeleton of a Choctaw woman who was 30 to 40 years old when she died in the early 1800s. "It was me," says Lippert. "It was so devastating to see. Over 100 years ago, I would have been dug up and put in a museum."

That personal connection sets Lippert apart from non-Indian archaeologists, she says. She and the small but growing number of other Native American archaeologists sometimes find themselves torn between their culture and their profession, as tribal traditions clash with scientific inquiry. Now new government rules, which allow tribes to claim "unaffiliated" remains and objects, are likely to stir up old tensions, as researchers

Bridging communities.

Stanford's Michael Wilcox sees growing interest in archaeology among Native Americans.

fear a loss to science (see main text, p. 166).

But as the original law ordering institutions to give back Native American bones celebrates its 20th birthday, Lippert and her colleagues are testimony to what has changed. In 1990, there were almost no Native Americans with Ph.D.s in archaeology, according to an informal tally kept by archaeologist Michael Wilcox of Stanford University in Palo Alto, California, who is a descendant of Arizona Yumans. Today there are 15 to 20, plus perhaps hundreds of Native American archaeologists without degrees or with master's, bachelor's, or associate degrees.

The central conflict these archaeologists face is how to deal with human remains, because cul-



tural and religious beliefs make handling them all but prohibited for many Native Americans. Some tribes, such as the Navajo, believe that contact with human remains can cause physical illness. Others respect the dead by not handling them and so avoid working with bones "out of consideration for our relatives," says Sonya Atalay, an Ojibwe and archaeologist at Indiana University, Bloomington. Wilcox recalls at first being interested in studying bones, but after working in a lab he grew troubled by the lack of sensitivity among his non-Native colleagues and switched to archaeology. How many Native

CREDITS (TOP TO BOTTOM): JAMES RIDING IN; LINDA A. CICERO/STANFORD NEWS SERVICES

the 1980s to 2006 and found that the number of anthropology theses using skeletal remains worldwide increased dramatically, while the percentage of U.S. work using Native American remains dropped sharply. She thinks students are abandoning research in North American bones in favor of greener pastures elsewhere (see sidebar, p. 170). Kakaliouras also found a steep drop in the number of papers based on Native American skeletal remains at annual physical anthropology meetings, but her results don't quite fit with Weiss's: She found that the decline didn't begin until after 2004 and thinks it may be due more to fear of NAGPRA than to NAGPRA itself.

The most comprehensive attempt to understand NAGPRA's impact is now under way at the University of Arizona in Tucson, led by physical anthropology Ph.D. student Elisabeth Cutright-Smith. She and two other graduate students are analyzing the content of two journals that represent different though related disciplines—the archaeological journal *American Antiquity* and the *American Journal of Physical Anthropology*—before and after NAGPRA. Like the other studies, the team found that worldwide analyses of human remains have risen since the 1970s.

But preliminary results show contrasting patterns in the two journals. The number of papers on Native American remains published by the archaeological journal increased after the early 1990s, but the number published by the physical anthropology journal began to decline in 2001. In addition, mention of consultation with tribes rose after 1996

Boning up. Anthropologist Elizabeth Weiss seeks to learn from ancient bones.

in *American Antiquity*, as did the amount of ethnohistorical data and oral tradition. But in the anthropology journal, use of tribal knowledge declined. “The passage of NAGPRA has provoked contradictory outcomes,” says Cutright-Smith.

Determining the law's effect on fieldwork is even more difficult to quantify. Under NAGPRA, archaeologists and land managers must notify tribes of finds on tribal or federal land, triggering what can be a protracted consultation. Utah's state archaeologist, Kevin Jones of Salt Lake City, says land managers find the NAGPRA process “daunting and burdensome” and “want a quick decision,” so they're inclined to swiftly turn newly discovered human remains over to tribes. “I have no doubt that information is being lost,” he adds.

Some archaeologists will even turn their backs on bones found in the field. Wilcox recalls finding a human bone in a national park with a National Park Service archaeologist, who immediately warned him against picking it up or even looking at it. That's not an option for those who rely on bones for data. “Archaeologists in the field can just avoid burials” and work on artifacts, explains Kakaliouras. “But for physical anthropologists, this is their bread and butter.”

Collaboration or conflict?

For many archaeologists, however, the loss of data that comes with repatriation is



trumped by its human-rights value. “Absolutely, we lose some pretty important information,” says Lekson. “But it's still the right thing to do.” And NAGPRA has actually proved beneficial to some researchers. “For a long time, indigenous people were left out of the equation,” says George Nicholas, an American archaeologist who teaches at Simon Fraser University in Burnaby, Can-

Americans handle Native bones as physical anthropologists today? Says Wilcox, laughing: “I would say zero.”

And yet archaeologists in the field are bound to encounter human remains at some point. “I'm faced with that all the time,” says Atalay, who researches the cooking practices of 9000 years ago by studying food residues in hearths and cooking vessels. Most of her “dirt archaeology” takes place at an ancient site in Turkey, where she excuses herself when human remains are excavated. John Norder, a member of the Spirit Lake Dakota Nation and a professor of archaeology and ethnohistory at Michigan State University in East Lansing, has done the same on past digs and says his “colleagues have been understanding.”

A legacy of exploitation colors the way Native American archaeologists are perceived both by their peers and by American Indians. In the early 1990s, a few years after the Native American Graves Protection and Repatriation Act was

Personal touch. Dorothy Lippert feels a connection to Indian remains.

enacted, Norder says Indian activists “would literally yell in my face” for being an archaeologist. Although passions have since cooled, Wilcox says Native American archaeologists are still considered suspect by the Indian community: “To them, it's like a chicken working for Colonel Sanders.” Lippert, who like Norder caught grief from Native American activists years ago, today gets an angry earful from some of her fellow archaeologists, who wonder where her loyalties lie because she supports the new rules. Says Wilcox wryly: “We manage to make everyone unhappy.”

Still, for Lippert at least, living in two worlds means she is positioned to make a difference. In July, 7 years after she learned about the bones, the female Choctaw skeleton was repatriated and reburied in Oklahoma.

—KEITH KLOOR

Keith Kloor is a writer in New York City.



A World of Graves

The United States isn't the only nation grappling with native claims on ancient skeletons (see main text, p. 166). But different cultures have wildly divergent views of their long-dead ancestors, with profound consequences for the kinds of research that can be done. Here's a brief roundup of world attitudes toward the study of ancient human remains:

Australia: As in the United States, tens of thousands of indigenous remains were collected by mostly white researchers 100 to 200 years ago. Today, laws allow—but do not mandate—return of Aboriginal bones to their descendants. In recent years, Aborigines have focused on recovering remains from U.S., British, and other foreign collections. Some 1000 were returned between 1998 and 2008.

Israel: Ultra-Orthodox Jews, offended by scientific study of human remains, have long argued for their immediate reburial. In 1994, in the wake of protests by this group, the attorney general ruled that archaeologists must turn over ancient bones to the Ministry of Religious Services for reburial. In September, grave finds at a Jaffa construction site sparked protests in the United States as well as in Israel.

Canada: No comprehensive legislation has been passed, but the country has a long-standing policy to repatriate remains and objects to its indigenous peoples, called First Nations, and there is a tradition of cooperation rather than antipathy between archaeologists and First Nations people. For example, one well-preserved 500-year-old corpse, found in a British Columbian glacier in 1995, was studied with tribal approval and cremated a decade later, after researchers had dated the bones, examined the clothing, and determined that the body's DNA resembled that of 17 people living nearby.



United Kingdom/Europe:

Studies of domestic ancient remains are welcomed here, as seen in the enthusiastic embrace of scientific work on Ötzi, the 5000-year-old Ice Man found in the Alps. But many museums have drawn fire for their large collections of bones from former European colonies; only half of the 20,000 human samples now in London's Natural History Museum

are British in origin. Although the prime ministers of Britain and Australia agreed in 2000 that Britain would return its Aboriginal remains, many museums have balked, with a few exceptions. The head of the Aboriginal warrior Yagan, killed by white settlers nearly 2 centuries ago, was returned and reburied in July in Western Australia.

In other parts of the world, there is often little difficulty in studying ancient bones. In East Asia, for example, burial practices today center on cremation, so living people feel little connection to ancient remains. In Africa, widespread pride in the continent's role as the cradle of the human species fosters positive attitudes toward paleontology.

But some physical anthropologists worry that indigenous tribes in South America will eventually focus on the issue, blocking the one open avenue for no-holds-barred studies of ancient Americans. In Chile, for example, a New Zealand team excavating on the west coast encountered concern among local tribes about how any bones might be treated (*Science*, 11 June, p. 1344). The result, worries physical anthropologist Elizabeth Weiss of San José State University in California, could be a stampede to more favorable places outside the Americas. "Those who want to study human remains," she predicts, "will go to Europe, where this is not going to be an issue."

—A.L.

ada. "Now archaeologists increasingly are working with descendant communities" in both the United States and Canada.

Collaboration can provide access to tribal knowledge and shed light on material remains, particularly from more recent eras. For example, Sebastian LeBeau, a Lakota who now teaches ethnic studies at Minnesota State University, Mankato, was able to elicit data on sites used for vision quests from the people of the Cheyenne River Reservation for his anthropology Ph.D. That gave researchers a more nuanced understanding of the way the people used the landscape, says Zimmerman.

Such work reflects the growing interest in archaeology within Indian communities. Wilcox believes that Native Americans are more curious now about the traditions of their ancestors, including diet, technologies, and material culture. "When you get beyond the politics, people are interested in these kinds of questions," he says. "Not the whole community, but enough to establish good relations." There are more Native American professors and students, as well as many who work for private cultural resource management companies, some of which are wholly owned by Native Americans. "Before this dialogue started, many Native Americans may have hated archaeologists," says Wilcox. "Now they see what it can do, that it can offer jobs, and they have a much more positive feeling."

On the scientists' side, with NAGPRA no longer an adolescent, a younger generation of researchers says they view the legislation as a historical fact and a welcome righting of past wrongs. Nicholas, for example, simply accepts that Native Americans have the right to withhold objects that contain what amounts to proprietary data. And he's confident that the new rules won't prove as damaging as many fear. "Some folks thought NAGPRA marked the end of archaeology," he says. "But 20 years later, it is more vibrant and relevant than ever."

Others, particularly physical anthropologists, are more pessimistic. "The next few years will be pretty dismal," predicts Weiss. "We'll see an increase in lawsuits, infighting by tribes, and more collections placed off-limits. It's not going to be a pretty picture." Riding In of ASU agrees there are dark clouds ahead. "Relations are still strained, especially with those hard-core scientists who want to deny Indians our human rights," he says. "They are just as determined to resist as we are."

—ANDREW LAWLER

With reporting by Keith Kloor, a writer in New York City.

CREDITS (TOP TO BOTTOM): IAN HODGSON/REUTERS/NEWSCOM; FRANCO ROLLO/AFP/GETTY IMAGES/NEWSCOM



A Tale of Two Skeletons

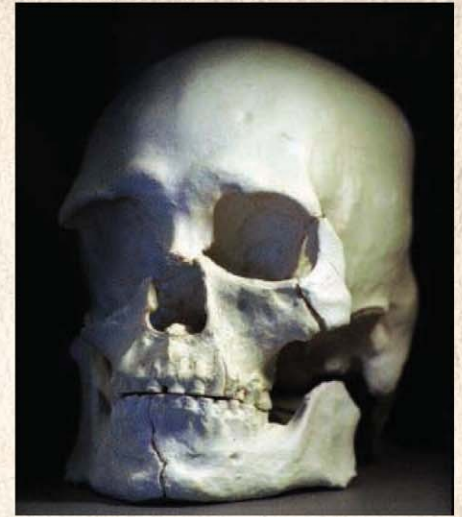
Researchers and Native Americans may be headed for clashes over ancient Paleoindian remains. But in at least one instance, the two communities collaborated—and produced good data to boot

When a team of archaeologists and paleontologists came across ancient human bones in a dank Alaskan cave in 1996, they immediately told members of the local Tlingit tribe. “They knew the community,” explains Rosita Worl, president of Sealaska Heritage Institute in Juneau. “And we gave them credit for understanding the human dimension of the find.” That initial openness sparked a remarkable 12-year-long collaboration between Native Americans and scientists to study the 10,000-year-old bones. The research project ended in reburial and a 2-day festival celebrated by Tlingit, scientists, and bureaucrats. “We gave talks, the elders spoke, and there were feasts,” recalls paleontologist Timothy Heaton of the University of South Dakota in Vermillion.

That story stands in marked contrast to that of a similarly old skeleton found the same year, the famous Kennewick Man. It was discovered by teenagers on the banks of the Columbia River in Washington state, just across the river from the Umatilla Indian Reservation on U.S. government property. A local archaeologist was called in, but there was no prior relationship between the Indians and scientists involved, and a spiral of suspicion and ill will began, observers say. Eventually the skeleton became the subject

of a bitter tug of war between scientists and the tribe, leading to a complex and expensive court battle that scientists won in 2004. But unlike the bones from On Your Knees Cave in Alaska, Kennewick Man to date has failed to produce significant new data such as DNA.

New rules by the U.S. Department of the Interior are now putting the spotlight on the rare skeletons that date back to 8000 to 12,000 years ago, which researchers call Paleoindians. Because the bones are so old, many scientists argue that they can’t be related to contemporary tribes, whereas Native Americans cite oral traditions claiming long ties to their land. The new regulations give tribes the right to any remains from traditional tribal lands (see main text, p. 166). Whether that



Poles apart. Officials celebrate the end of Alaskan collaboration, whereas Kennewick Man (right) sparked a bitter court battle.

will spark renewed attempts by Native Americans to rebury these ancient dead remains to be seen, but scientists are worried. “There are so few of them,” physical anthropologist Elizabeth Weiss of San José State University in California says of the few dozen Paleoindian skeletons. “Each one is precious.”

At stake are answers to what is arguably the greatest question of American prehistory: Who settled the Americas, when, and how? In the past decade, many researchers have given up the traditional view that big-game hunters from the Clovis culture were the first arrivals some 13,000 years ago. Researchers now envision an earlier and more complicated series of migrations from Siberia to Alaska. Given advances in DNA techniques, anthropologists think Paleoindian skeletons may hold clues to how the migration unfolded as well as the origin of the first Americans.

To date, advances in understanding the settlement of the Americas have come largely from archaeological data rather than ancient skeletons. Even much of the DNA evidence comes from living people. But better and cheaper methods for extracting ancient DNA are making it possible to glean new data from



Status of Paleoindian Remains

- | | | | |
|---------------------------------------|--|--|---|
| 1. Kennewick, WA
Not available | 6. Arlington Springs, CA
Unclaimed | 11. Hour Glass Cave, CO
Reburied | 15. Wet Grave, Lime Creek, Swanson Lake, and Gilder Mound, NE
Reburied |
| 2. Prospect, OR
Reburied | 7. UCSB, San Diego, CA
Status unclear | 12. Gordon Creek, CO
Status unclear | 16. Browns Valley, MN
Reburied |
| 3. Wizards Beach, NV
Not available | 8. Whitewater Draw, AZ
Unclaimed | 13. Horn Shelter, TX
Unclaimed | 17. Pelican Rapids, MN
Reburied |
| 4. Spirit Cave, NV
Not Available | 9. Buhl, ID
Reburied | 14. Wilson-Leonard, TX
Unclaimed | |
| 5. Grimes Point, NV
Not available | 10. Anzick, MT
Status unclear | | |

the old bones. “There is a wave of new information coming,” says physical anthropologist Michael Waters of Texas A&M University in College Station. “We’re right on the cusp.”

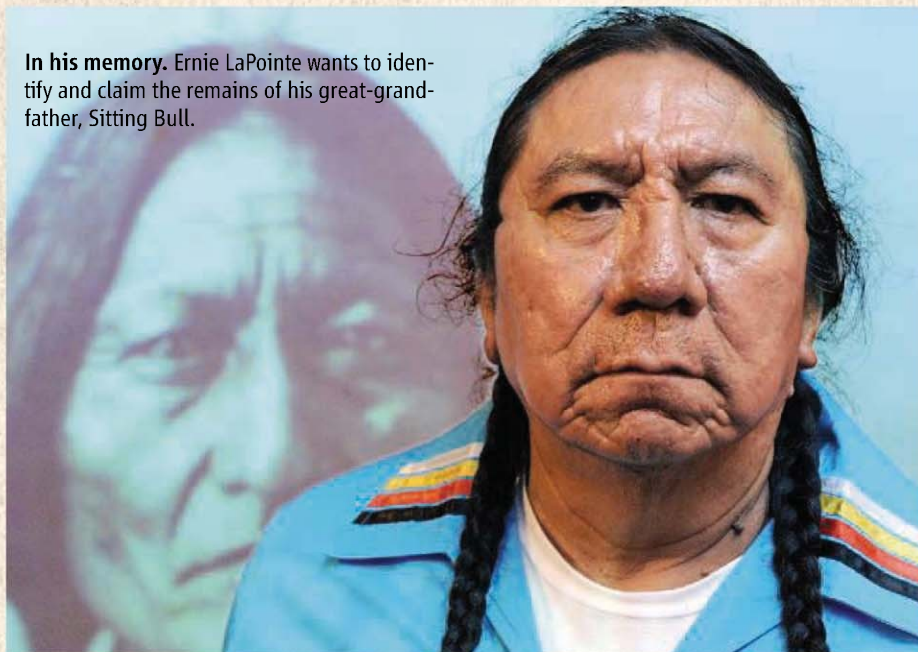
In the case of the Paleoindian found in On Your Knees Cave, some 200 Tlingit signed letters of consent to have their DNA tested to see if it matched that of the skeleton. No match was found, says physical anthropologist Theodore Schurr of the University of Pennsylvania, but the bones did carry DNA patterns that match those of living Native populations centered on the west coasts of North and South America. That suggests that in at least one migration wave, people moved southward along the coast or in boats.

Of the other ancient remains, some, such as those of Kennewick Man, are too degraded to produce DNA. And others are not accessible to science (see map, p. 171). A 9000-year-old skeleton found in Minnesota was reburied by Native Americans in 1999, as was one from Hourglass Cave in the Colorado Rockies, both over objections from researchers. Other remains, such as those found in Spirit Cave, at Grimes Point, and on Wizards Beach—all in Nevada and all 9000 years old or older—are locked away pending legal decisions. And the remains of an 18-month-old dubbed Anzick, who lived nearly 11,000 years ago in Montana and is the only person associated with the Clovis culture, have been only partially studied, even though they were found on private land and so are not subject to repatriation rules. Although Montana recently declared Anzick’s skeleton a “state treasure,” its fate is nevertheless uncertain, says anthropologist Dennis Stanford of the Smithsonian Institution in Washington, D.C.

Heaton, after his experience with the Alaskan cave, says that a more collaborative approach may yield greater dividends than expensive legal battles. That requires compromise. In the case of the On Your Knees Cave remains, for example, “it would have been nice to have had the bones forever,” because more data can be extracted as techniques improve over time. “But [the Tlingit] gave us plenty of time and respected our wishes,” he says. “Each side gave in to some degree.” Worl agrees. “This went from cooperation to collaboration.” Tribal members even worked on the dig.

Tlingit and scientists say they ultimately shared similar goals. “We felt this was an ancestor offering himself so we could learn from him,” Worl says. She cites the Tlingit concept of *haa shagoon*—that ancient, present, and future generations are linked—as a way to link tribal traditions to scientific rigor, for the benefit of both communities. —A.L.

In his memory. Ernie LaPointe wants to identify and claim the remains of his great-grandfather, Sitting Bull.



In Search of Sitting Bull

Can DNA help Sitting Bull’s great-grandson prove his heritage and find the warrior’s remains—while giving science a rare glimpse of an early Native American’s genes?

In 2007, ancient DNA expert Eske Willerslev found himself sitting in a pitch-black basement in South Dakota, participating in a Native American ceremony involving a medicine man, singers, drummers—and Ernie LaPointe, the great-grandson of famed Lakota warrior and holy man Sitting Bull, who defeated George Custer at the Battle of Little Bighorn. At the request of LaPointe, Willerslev had traveled from his home in Denmark to take a snippet of Sitting Bull’s hair for DNA analysis. LaPointe hopes to use the genetic information to confirm his heritage—many people claim to be related to Sitting Bull, including someone in Denmark, notes Willerslev—and to identify, claim, and perhaps rebury the chief’s remains. For Willerslev, DNA from Sitting Bull’s hair, and his remains if conclusively identified, could shed new light on the genetics of early Native Americans and the peopling of the New World.

But before this unusual collaboration could happen, LaPointe said the basement ceremony was required to get permission from Sitting Bull himself, who was killed in 1890. Willerslev says something odd happened at the ceremony, recalling a blue-green light that ran across his body and into his

mouth. LaPointe says the spirit of Sitting Bull tested the geneticist and approved. As a result, LaPointe allowed Willerslev to cut a short section from a long braid of Sitting Bull’s still-shiny black hair and fly it back to the University of Copenhagen for analysis.

Three years later, Willerslev is still analyzing what turns out to be badly damaged DNA from the hair. Sitting Bull is so revered that any results may stir strong feelings in a community that has been distrustful of scientists seeking to study the remains of their ancestors (see main text, p. 166). Yet the tale of a cutting-edge geneticist and a Native American working together on the chief’s remains may also suggest a way forward. “I hope this initiates better collaborations between Native Americans and geneticists,” Willerslev says optimistically and perhaps naively.

The tangled tale of the war chief’s remains includes elements common to other cases of repatriation of remains, including grave desecration and tragic divisions among Native Americans. A leader of resistance against the U.S. government, Sitting Bull was killed during a botched arrest by Lakota tribal police at Standing Rock Reservation in South Dakota. His body was buried in Fort Yates, a military outpost in North Dakota on the reser-

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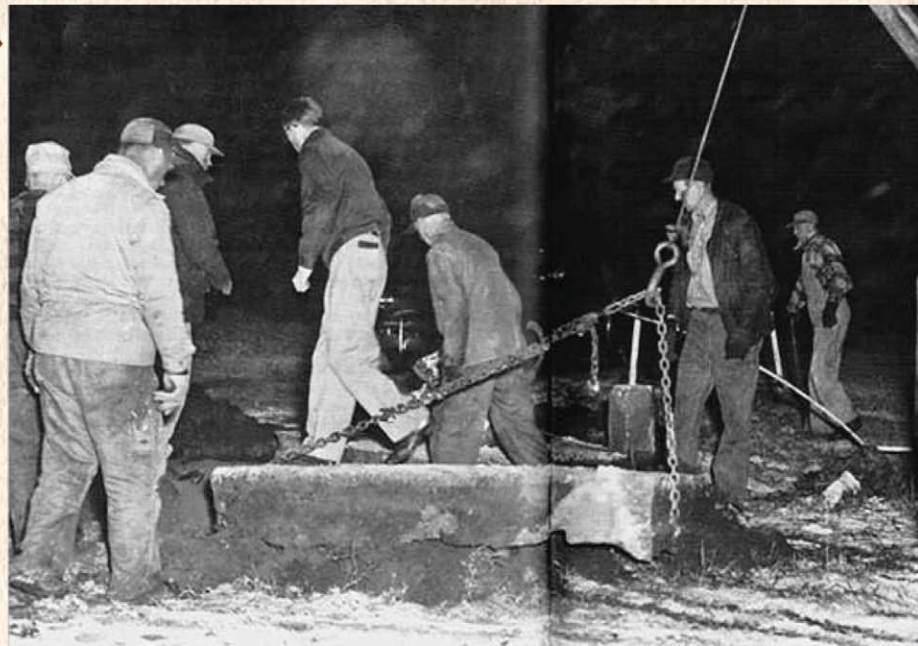
S For an extended conversation with Ernie LaPointe about his ancestor Sitting Bull and the project to analyze his DNA, see *Science* Online.

Removed. In 1953, Sitting Bull's remains were moved to a new location on reservation land.

vation. Decades later, North Dakota denied requests to move the remains nearer to Sitting Bull's home. So in the middle of the night in 1953, Native Americans (including a young LaPointe and his mother and aunts) and South Dakota citizens dug up what they believed were Sitting Bull's remains and reburied them on Standing Rock land in South Dakota. North Dakota officials, however, said the group took the wrong bones. And some tribal officials said later that the bones had been moved yet again from the public, often vandalized, gravesite in South Dakota, says LaPointe. "There are many claims that he's buried in many places," says William Billeck, head of the repatriation office at the Smithsonian Institution's National Museum of Natural History (NMNH) in Washington, D.C.

The museum and Billeck entered this saga in 1999 when NMNH discovered among its unexhibited collections leggings and a scalp lock, a long braid worn by many Native American warriors to hold up their eagle feathers. The two items were accompanied only by a note saying "taken from Sitting Bull." Billeck learned that the items had been loaned to the museum in 1896 by a physician who had examined Sitting Bull's body. Billeck decided that under the Smithsonian's repatriation laws (which are separate from but roughly parallel to national laws), the museum should return the objects. The Standing Rock tribe, a member of the larger Lakota tribe, put in a claim, but genealogical records and other documents convinced Billeck that LaPointe and his sisters were the closest direct kin to Sitting Bull. Lineal descendants have priority over tribal claims in repatriation laws. So in 2007, the Smithsonian returned Sitting Bull's hair and leggings to LaPointe.

Around the same time, Willerslev read about the repatriation plans and LaPointe's battle to identify and claim his ancestor's remains. When the bones were taken in 1953, LaPointe says, his mother was outvoted by her sisters on where to rebury them and hadn't wanted Sitting Bull buried on the land of those who killed him. Confident that the remains in the 1953 grave are those of his ancestor, LaPointe is fighting to get them back. He believes Willerslev's DNA analysis will help persuade South Dakota to give him the remains, even if Standing Rock officials object. "That will solidify our connection to our great-grandfather.



No one can say we are unrelated." LaPointe says Willerslev could then test the remains from the 1953 site to confirm they're Sitting Bull's. If so, LaPointe plans to rebury them in a private grave.

For Willerslev's part, he's excited at the prospect of reading the nuclear genome of a pure-bred Native American from North America; most historical or ancient Native Americans studied genetically have been from South America. The hair analysis has gone slowly, as his team has had to develop methods to separate the DNA from unknown chemicals or pesticides, perhaps applied to protect the hair, which have also damaged it. So far, the hair's mitochondrial DNA has genetic markers clearly identifying it as Native American—a relief, given the number of people who have handled it. Still, Willerslev isn't sure the hair will yield a full nuclear genome.

For now, he plans to try to read 14,000 specific DNA bases that can vary from person to person. A scan of these single-nucleotide polymorphisms should provide enough of a DNA fingerprint to confirm that LaPointe is related to the hair's owner and also to identify any remains as Sitting Bull's. LaPointe is helping Willerslev get DNA from other living Lakotas for comparison.

Other researchers agree that the project has scientific promise. "The potential for this is fantastic. It's just a single genome, but as a reference, it's valuable," agrees biological anthropologist Keith Hunley of the University of New Mexico, Albuquerque. "As long as there is no European ancestry in that lineage, it's a window into the deep past." But Hunley is not sure he would dare to pursue this project himself, given the tensions between Native Americans and anthropologists. "I would be very leery," he says. "It could be one more example of scientists telling Native Americans what their history is."

Willerslev, who grew up admiring the legend of Sitting Bull and once had a poster of him, says he is acting with respect. "Sitting Bull is my biggest hero of all times; brave and wise. Thinking of others rather than himself. The perfect leader," Willerslev says. He notes he didn't protest—much—when LaPointe gave him only a small sample of hair and burned the rest.

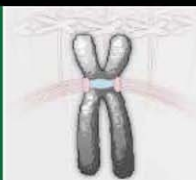
LaPointe also isn't worried about controversy, trusting in the wisdom of Sitting Bull. "If he said give him the hair, I'm not going to argue."

—JOHN TRAVIS



At work. Eske Willerslev is analyzing ancient DNA from Sitting Bull's hair.

CREDITS (TOP TO BOTTOM): MORRIDGE TRIBE/NE TINA B. BRANT, CENTRE FOR GEOGENETICS



LETTERS

edited by Jennifer Sills

AIDS Funds: Promised

IN THEIR POLICY FORUM “GLOBAL HIV/AIDS POLICY IN TRANSITION” (11 June, p. 1359), J. Bongaarts and M. Over argue that expansion of HIV treatment is unsustainable. In 2005, the G8 promised Africa \$25 billion in order to ensure “universal access” to HIV treatment by 2010 (1). The subsequent expansion of HIV treatment has paved the way for large-scale global health funding mechanisms. However, the G8 commitment to “universal access” is at least 30% short (2). The Global Fund donations from the United States are more than a billion dollars less than their fair share (3), and the funding for the U.S. President’s Emergency Plan for AIDS Relief has increased this year by the smallest increment in the program’s history (4). Rather than scaling down antiretroviral therapy as Bongaarts and Over suggest, we should ensure that the world lives up to its promises.

Bongaarts and Over’s failure to mention the human cost of treating only “a percentage...of those newly needing treatment” is indicative of how cost-effectiveness can be used to avoid fulfilling one’s commitments and promote substandard care, such as the authors’ support for initiating antiretroviral therapy at CD4 levels well below current World Health Organization guidelines. Moreover, the emphasis on pure cost-effectiveness only serves to divert attention from the

real issue of what Girard and colleagues correctly term a need for “an urgent increase in global health funding” (5). As the world strives to make the global funding pie bigger, discussions should center on how to increase the resources available and the most efficacious use of those funds. Instead of working to “transition” away from HIV treatment programs that have been proven to reduce transmission by 92% (6) and have brought hope to countries once thought beyond saving, let us concentrate on how to best advocate for more funding and better policies that allow the world to fulfill its commitments and ensure the right to health for the world’s most vulnerable.

DONNA BARRY* AND MEGAN TOWNSEND

Partners in Health, Boston, MA 02215, USA.

*To whom correspondence should be addressed. E-mail: dbarry@pih.org

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AIDS Funds: Undervalued

IN THEIR POLICY FORUM “GLOBAL HIV/AIDS policy in transition” (11 June, p. 1359), J. Bongaarts and M. Over argue that the costs of AIDS funding—especially for antiretroviral treatment (ART)—are excessive and imply that funding should be redirected to other health priorities. Their arguments are misleading.

Bongaarts and Over argue that HIV/

AIDS spending is “disproportional” to the disease burden. Yet if we look at the share of spending in relation to disability-adjusted life years (DALYs) lost due to AIDS (1), it is clear that in many countries—especially southern Africa, where most HIV-positive people live (2)—the overall share of the total national health resources (3) that is spent on AIDS (2) is lower than the contribution made by AIDS to the burden of disease (1) (see the figure, opposite page).

Bongaarts and Over also argue that ART costs are unsustainable, but they substantially overestimate the funding required. Their costing model assumes that drug prices will remain constant (when they are likely to fall as the market expands) and that ART has no effect on HIV prevention despite evidence that ART reduces infectiousness. They also adopt a very narrow concept of cost-effectiveness, which fails to consider indirect benefits and cost-saving (e.g., they look at the costs of mother-to-child transmission preven-

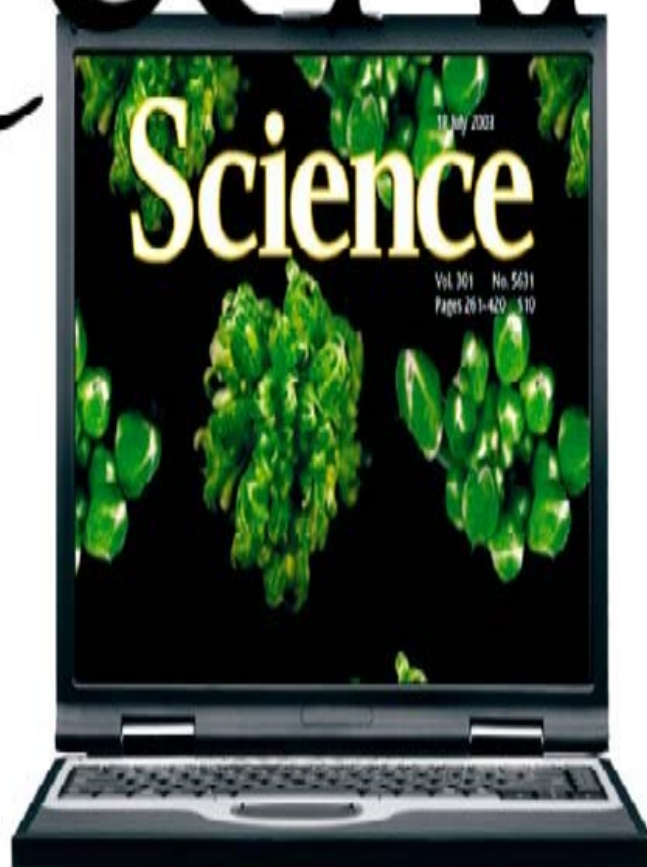
tion programs in isolation of the cost-savings obtained by reducing HIV rates). They overlook studies that take into account the medical costs of averted AIDS-related opportunistic infections. These studies have shown substantially reduced net costs—with some Brazilian and South African studies showing that ART programs can actually be cost-saving overall (4–6).

Bongaarts and Over criticize AIDS funding for being inefficient and undermining health systems, but their evidence is biased. They cite a negative World Bank evaluation of its African AIDS projects (7) (which were predominantly emergency operations in unstable environments), but they ignore the many more-successful AIDS interventions funded by the Global Fund (8). There are many positive examples of synergies between AIDS programs and health systems development (9–12). A growing body of evidence suggests that services to address HIV can strengthen health systems by train-

Letters to the Editor

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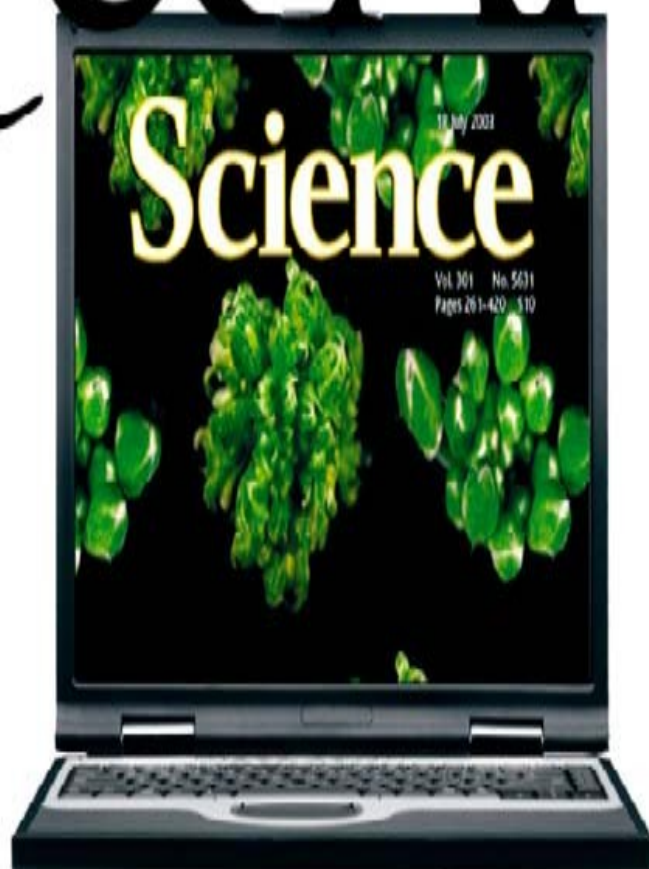
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Nanoparticles take on protein functions

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A corn halo

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ing personnel [p. S30 in (13)], improving drug procurement and distribution systems [p. S34 in (13)], enhancing the role of nurses and community workers in health service delivery [p. S20 in (13)], involving community in research and service delivery beyond HIV [p. S63 and p. S67 in (13)], and developing electronic health records [p. S54 in (13)].

The real obstacle is the political landscape of health policy. Where there are no clear patterns of accountability and incentives, health policy implementation is doomed to failure (7, 14). In the absence of easily measurable outputs and clear, politically feasible and sustainable mechanisms to hold government to account, funds can all too easily be misappropriated (15) or shifted away from priority health interventions—as was the case in Zambia when the TB program collapsed after being “integrated” into the general health care system (16).

AIDS spending, by contrast, can be linked to specific targets and has a constituency (treatment activists) with a strong incentive to hold governments accountable. Moreover, precisely because ART patients need standard health care as well as specialized HIV services, they have an incentive to ensure that both are efficient and effective. Undercutting HIV funding, ostensibly in order to build a

better health care system, could dismantle the most organized and effective health care consumer constituency in existence in developing countries.

NICOLI NATTRASS^{1*} AND GREGG GONSALVES²

¹Yale University and AIDS and Society Research Unit, University of Cape Town, South Africa. ²Yale University and International Treatment Preparedness Coalition.

*To whom correspondence should be addressed. E-mail: nicoli.nattrass@gmail.com

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AIDS Funds: Benefits

IN THEIR POLICY FORUM “GLOBAL HIV/AIDS policy in transition” (11 June, p. 1359), J. Bongaarts and M. Over contend that global health initiatives focused on HIV/AIDS treatment, such as the U.S. President’s Emergency Plan for AIDS Relief (PEPFAR), are not the best use of international health aid. We disagree.

Bongaarts and Over suggest that funding for HIV/AIDS is disproportionately high despite comprising <5% of the mortality burden in the developing world. However, in many countries death certificate misclassifications underestimate mortality attributed to HIV/AIDS by as much as 50% because the cause of death is often classified as an AIDS-related opportunistic infection (including tuberculosis, pneumonia, and meningitis) without reference to HIV (1).

Next, Bongaarts and Over propose that HIV/AIDS “distorts health priorities.” In Haiti and Rwanda, where adult HIV prevalence is <3% and the highest causes of morbidity and mortality are attributed to diseases other than HIV, global health initiatives focused on providing HIV treatment have directly and indirectly strengthened primary healthcare systems beyond HIV/AIDS, including maternal and child health (2–4).

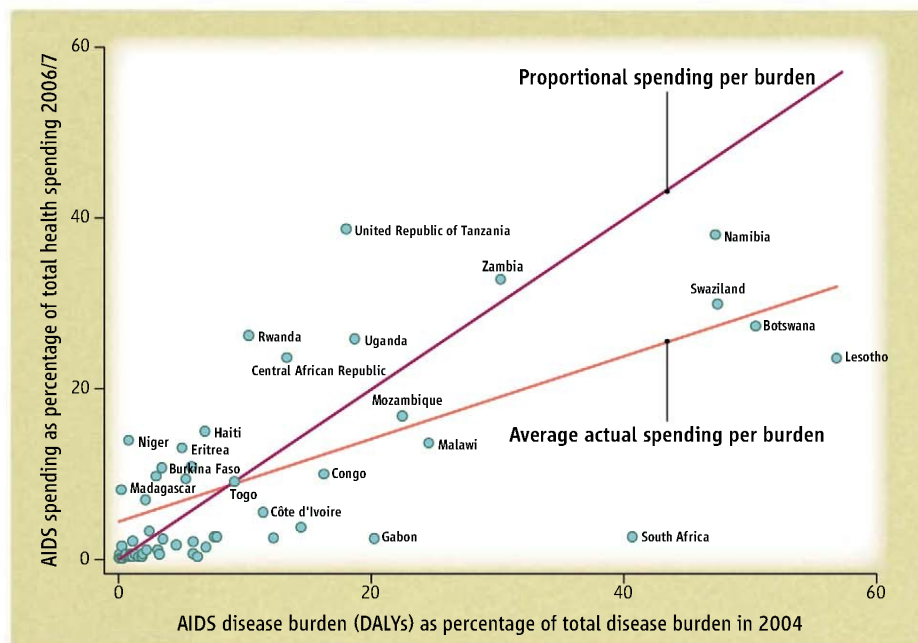
The authors claim that HIV treatment scale-up is not cost-effective. Several studies demonstrate that failure to use HIV/AIDS antiretroviral treatment is even more expensive than the provision of medication (5–7). Walensky and Kurtzkes emphasize that cost-effectiveness analysis may not be appropriate without considering the context; the treatment, population, and program must be comparable for conclusions to be drawn (8).

Finally, Bongaarts and Over argue in favor of HIV prevention over treatment. This logic contradicts recent studies suggesting that HIV/AIDS antiretroviral treatment may actually serve an important role in preventing new HIV infections, both by lowering viral loads in HIV positive persons and by encouraging people to present for voluntary counseling and testing (9, 10).

ANAND REDDI^{1*} AND SARAH C. LEEPER²

¹University of Colorado, School of Medicine, Aurora, CO 80045, USA. ²Brown University Medical School, Providence, RI 02906, USA.

*To whom correspondence should be addressed. E-mail: anand.reddi@gmail.com



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AIDS Funds: Rwanda

IN THEIR POLICY FORUM "GLOBAL HIV/AIDS policy in transition" (11 June, p. 1359), J. Bongaarts and M. Over conclude that anti-retroviral therapy for AIDS is not a cost-effective use of donor funding for global health. We believe that Bongaarts and Over omitted key aspects of a complete cost-effectiveness assessment: numerous positive spinoffs to other services, the effect on increased attention on global health, and the ability to mobilize resources that would not otherwise have been available for global health.

The situation in Rwanda serves as an example. Rwanda's total health expenditure declined from 1998 through 2002, but because of advocacy for HIV resources, total health expenditure doubled between 2002 and 2003 (*1*). HIV funding, even before the current focus on integration, provided resources for the renovation of health facilities, equipment, training, and management, as well as a culture of openness for best practices. With integration of HIV services into other health services, resources have had an even wider benefit, especially for maternal health services. Integration is still in progress, but successful steps have been made (*2, 3*).

Rwanda's 2006 National Health Accounts reported that although reproductive health spending did not grow as rapidly as overall spending, it increased by 15% between 2002 and 2006 (*4*), and maternal mortality rate decreased from 1071 (in 2000) to 750 per 100,000 births (in 2005) and is expected to fall to about 350 in 2010. Rates of assisted deliveries, use of family planning, and receipt of four standard prenatal care services have seen a marked increase (*4–6*). Consistent with the results in Rwanda, a recent *Lancet* article correlated maternal mortality with HIV mortality in women (*7*). These improvements cannot be attributed to reproductive health spending alone; major health initiatives made possible by HIV funding are largely responsible.

HIV resources also paid for the community-based health insurance premiums of many of the poorest segments of the

population; the funding thus contributed to the increase in community-based health insurance enrollment rate from 7% in 2003 to 85% in 2008.

The literature cited by Bongaarts and Over failed to consider all benefits gained beyond direct HIV services, including the value of saving a mother and its contribution to quality of life for her children and family and societal productivity. In doing so, Bongaarts and Over have greatly underestimated the impact of HIV funding on both HIV and non-HIV health results.

ANITA ASIIMWE,¹ ANGÉLIQUE K. RWIYEREKA,^{2*}
JOAN A. KAUFMAN,² DONALD S. SHEPARD²

¹Rwanda National HIV/AIDS Control Commission, National AIDS Commission, Post Office Box 7162, Kigali, Rwanda.

²Schneider Institutes for Health Policy, The Heller School, Brandeis University, Waltham, MA 02454, USA.

*To whom correspondence should be addressed. E-mail: gasugi@gmail.com

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AIDS Funds: Prevention

WE ARE INTRIGUED BY THE PROVOCATIVE Policy Forum "Global HIV/AIDS policy in transition" (11 June, p. 1359), in which J. Bongaarts and M. Over reconsider the balance between global health funding for HIV treatment and HIV prevention. The authors rightly emphasize the need to boost prevention efforts. Sustainable scale-up of antiretroviral therapy (ART) requires more effective prevention. To further stretch our prevention dollars, the U.S. President's Emergency Plan for AIDS Relief (PEPFAR) supports the authors' call for greater reliance on cost-effective interventions. In addition, it is imperative that we focus our efforts and funds on those prevention services with the greatest demonstrated impact, such as male circumcision, effective behavioral interventions, and prevention of mother-to-child transmission (PMTCT), and that we ensure that new prevention programs include impact evaluations. However, several important considerations overlooked by Bongaarts and Over serve as arguments in favor of maintaining substantial funding for AIDS treatment.

By quoting global rates of HIV, Bongaarts and Over underemphasize the enormous bur-



den of HIV in sub-Saharan Africa, where 67% of the people infected with HIV live (60% of whom are women), and where HIV is the leading cause of death among adults (*1*).

To argue for a substantial reduction in the pace of ART scale-up, Bongaarts and Over use cost-effectiveness estimates from the Disease Control Priorities Project (DCPP) (*2*) that were generated 5 to 6 years ago. ART costs today are in many cases less than one-half of what they used to be, due in large part to efficiencies gained in service delivery and commodities prices (*3–5*). Bongaarts and Over's estimate also omits the use of antiretroviral drugs (ARVs) for the prevention of vertical HIV transmission (PMTCT), which the same report lists as having a cost per disability-adjusted life year saved as low as \$34. PEPFAR is currently making substantial additional investments in PMTCT, an intervention that when provided as full ART, saves the life of the mother and protects an infant from HIV infection and orphanhood.

The Policy Forum overlooks the important benefits of ART in reducing sexual transmission; results from randomized clinical trials published recently point to a 92% reduction in the rate of new infections from HIV-infected persons on treatment to their regular partners (*6*).

Treatment programs in Africa have shown convincingly that ART not only saves lives, but also restores the labor productivity of treated adults and improves the nutrition and educational outcomes of their children (*7–12*). The cost-effectiveness comparisons quoted by Bongaarts and Over fail to appropriately capture these benefits, which are particularly salient for ART compared with health interventions for other diseases because HIV/AIDS is a chronic illness that affects individuals in their most productive years.

The benefits from prevention and those from treatment also accrue across different time horizons. Benefits from resources used to prevent new infections will be realized years from now. Failing to scale up ART in the interim would mean a sizable reduction in national economic activity, many fewer healthcare workers and teachers, and worse educational outcomes for today's generation of children, in addition to the humanitarian impact of increased mortality (11, 12).

Finally, we agree that programs need to link treatment with prevention. However, given the challenges in scaling up prevention (13, 14), linking treatment dollars to the demonstration of effective prevention would be excessively proscriptive. Even so, it is clear that national governments in both low- and middle-income countries, as well as international partners, must set higher sights for intensified and more effective prevention in the coming years.

CHARLES B. HOLMES,^{1*} HARSHA THIRUMURTHY,²
NANCY S. PADIAN,¹ ERIC P. GOOSBY¹

¹Office of the U.S. Global AIDS Coordinator, Washington, DC 20522. ²The World Bank, Washington, DC 20433, USA.

*To whom correspondence should be addressed. E-mail: holmescb@state.gov

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Response

THE LETTERS BY BARRY *ET AL.*, NATTRASS *ET AL.*, Reddi *et al.*, Asiimwe *et al.*, and Holmes *et al.* dispute our conclusion that donor funds spent on AIDS treatment save fewer years of life than would the same resources allocated to prevention or to many other health problems of poor countries.

We share Barry *et al.*'s belief that donors should spend more than they currently do on improving the health of poor people and that,

other things equal, they should respect their prior commitments to such spending. However, in this era of financial crisis, insisting that tax payers in the developed world provide enough financing to pay both for universal access to AIDS treatment (as world leaders promised in 2005) and to fully fund all the more cost-effective health programs is unrealistic and does more harm than good for the health of the poor. When everyone else is forced to confront tradeoffs and prioritize expenditures, we in global health must do the same.

We expressed concern that AIDS consumes 23% of foreign assistance for health but causes less than 5% of deaths and disability-adjusted life years (DALYs). In response, Natrass *et al.* argue that we must consider allocated AIDS spending as a percentage of total health spending, not just as a percentage of health-related foreign assistance as we discussed. We agree, especially regarding South Africa, where AIDS spending has been notoriously unequal to the size of the problem. However, we have two reservations.

First, Natrass *et al.*'s UNAIDS estimate of AIDS spending (plotted on the vertical axis of their figure) excludes a large proportion of international AIDS funding. The amount of money donated to each country is reported by HIV/AIDS aid donors to the Organisation for Economic Co-operation and Development (OECD), and these figures are often much larger than the amounts that the country governments report having received to the U.N. (reflected in Natrass *et al.*'s UNAIDS data) (1, 2). In 2007, for the 16 Sub-Saharan countries available in the UNAIDS data, the donors report sending about twice as much as the recipients report receiving. The largest discrepancy was for Nigeria, which told the U.N. it received 8.4 million but appears in the OECD data to have received 213 million. To the countries of Botswana, Namibia, Senegal, and South Africa, donors claim to have disbursed more than the countries claim to have received by 70, 35, 82, and 102%, respectively. A key reason for this discrepancy is that a large part of foreign donor expenditures passes directly through contractors to AIDS patients without ever being reported to the government. If the donor-reported OECD data rather than the recipient-reported U.N. data are used, AIDS's share of total health spending is larger than AIDS's share of the disease burden in more countries than Natrass *et al.*'s figure suggests.

Second, regardless of data discrepancies, we believe that health spending should not be allocated in any strict proportion to disease burden, but rather in proportion to the marginal return in terms of reducing disease

burden. We advocate allocating incremental resources to the interventions that save the most life-years per dollar spent.

Reddi *et al.* believe the spending appears disproportionate because AIDS deaths are underreported in many countries. This is true, but we use internationally accepted UNAIDS estimates of AIDS deaths and HIV prevalence, which avoid this problem by modeling both quantities based on verifiable sero-survey data.

Asiimwe *et al.*, Natrass *et al.*, Holmes *et al.*, and Reddi *et al.* point out that AIDS treatment has beneficial effects on other diseases, on the health care system, and on labor productivity. We agree that these effects should be taken into account in allocating resources. However, the same logic applies to most other disease interventions. Clearly, various indirect benefits would also be available either for HIV prevention or for other competing, more cost-effective interventions. For example, performance-based financing in Rwanda has strengthened health-care delivery systems independently of whether AIDS treatment was part of the service delivery mix (3).

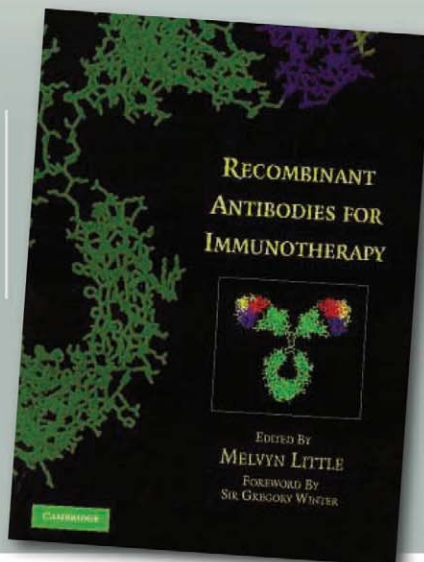
Natrass *et al.* and Holmes *et al.* note that the cost of ART drugs has decreased. However, the effects of greater competition have largely been exhausted for the last generation of first-line drugs. The next generation will cost more because they are still under patent. Even if the cost of drugs were to decline further, the related health care is more expensive than the drugs. PEPFAR estimates that a year of treatment now costs, on average, \$814, of which only 39% is for drugs (4). We strongly agree with Natrass *et al.* that cost-effectiveness should be the proper way to assess how to allocate funds. Given the continuing relative high cost of treatment, other health interventions and in particular HIV prevention should be given higher priority. In fact, prevention (including of vertical HIV transmission) is the only way to bring the epidemic under control.

As Natrass *et al.* and Holmes *et al.* discuss, ART makes HIV positive individuals less infectious. If all HIV-positive people could be treated as soon as they are infected, the epidemic would likely disappear eventually (5). Unfortunately, the cost of finding and treating tens of millions of people is prohibitive. The current goal of universal treatment for everyone with a CD4 count below the WHO threshold would have a much smaller impact on the epidemic because the majority of infections occur in the years between infection and the onset of treatment.

Like Natrass *et al.*, we are persuaded that political and financial support of health care

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LETTERS

improves with increases in the accountability of health care facilities and providers. But instead of using this as a reason to continue growing ART financing, we draw the lesson that HIV prevention and the other health interventions must increasingly be held to equally high accountability standards and that doing so will increase their political support.

We remain convinced that the current allocation of international health resources is far from optimal and that a future reallocation will improve the health and well-being of poor populations.

JOHN BONGAARTS¹* AND MEAD OVER²

¹Population Council, New York, NY 10017, USA. ²Center for Global Development, Washington, DC 20036, USA.

*To whom correspondence should be addressed. E-mail: jbongaarts@popcouncil.org

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6. We thank T. Velayudhan for valuable research assistance.

TECHNICAL COMMENT ABSTRACTS

Comment on "Climate, Critters, and Cetaceans: Cenozoic Drivers of the Evolution of Modern Whales"

Nicholas D. Pyenson, Randall B. Irmis, Jere H. Lipp

Marx and Uhen (Reports, 19 February 2010, p. 993) suggested that correlated diversity changes in the fossil record of whales and diatoms reflects secular evolutionary signals of underlying ecological drivers. We question the meaning of this association and outline avenues for more complete testing of correlations between productivity and marine consumers through geologic time.

Full text at www.sciencemag.org/cgi/content/full/330/6001/178-a

Response to Comment on "Climate, Critters, and Cetaceans: Cenozoic Drivers of the Evolution of Modern Whales"

Felix G. Marx and Mark D. Uhen

Pyenson *et al.* raise concerns about the correlation we identified between diatom and cetacean diversity through time. Although the issues raised are of investigative interest, they do not invalidate our conclusions. We agree that localized studies combined with global data sets will further our understanding of the factors that have helped shape cetacean evolution.

Full text at www.sciencemag.org/cgi/content/full/330/6001/178-b



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PHYSICS

One Theory to Rule Them All

Joseph Silk

In *The Grand Design*, Stephen Hawking, in collaboration with Leonard Mlodinow, addresses some of the biggest questions in the universe: When and how did it begin? Why is there something rather than nothing? Why are we here? And does the grand design of the universe provide evidence of a creator?

The core of the book describes an attempt to unify the fundamental forces of matter with the force of gravity. Quantum theory deals with the infinitesimally small, and gravity with the cosmically large. The quantum forces are strong, and gravity today is weak. Only at the beginning of the universe, in the Big Bang, was there an environment where the forces must have been comparable in strength. Attempts to unify gravity with quantum theory have attracted the brightest minds in physics, from Einstein onward.

The first serious attempts at the physics of unification developed via string theory, which describes elementary particles in terms of one-dimensional vibrations, rather like infinitesimal pieces of string as opposed to points. There were competing string theories, and a breakthrough finally came when it was realized that all were manifestations of an underlying theory called M theory.

M theory predicts the existence of ten spatial dimensions. We observe three. This ought to be enough for any rational person to say M theory conflicts with nature. String theorists are clever, however, and saved the theory by arguing that seven of the dimensions are now tightly curled up in the low energy limit. M theory succeeds in unifying gravity and quantum theories. It is the only current candidate for a complete theory of the universe, and Hawking sees it as the realization of Einstein's dream. Unfortunately, M theory has hitherto made essentially no testable predictions in the limit of low-energy physics, apart from the possibility that particle collisions in the Large Hadron Collider will spawn infinitesimal black holes as relics of higher dimensions.

Some physicists might prefer to move onto other theories. Indeed, most do not even recall

what the M means in M theory. This ought to provide an indication of the reality of the hyperspace inhabited by the theorists. To be fair, however, M theory does take one important step toward unification by removing the infinities that plagued earlier theories of quantum gravity. This is a compelling advance, and Hawking argues that its predictions must be taken seriously.

M theory predicts the existence of some 10^{500} universes, one of which we inhabit. This seems an improbable situation. Of course, the existence of life in the universe may also be highly improbable, especially

given the barrenness of our solar system and the bleak conditions on the 500 or so extrasolar planets discovered to date. M theory demonstrates that most of the predicted universes are unreconcilably hostile to life as we know it. They are simply too young, too chaotic, or have the wrong values of the fundamental constants of physics. The problem is compounded by the acceleration of the universe, produced by so-called dark energy—which itself is improbably small in strength compared to expectations from particle theory, by 120 factors of 10.

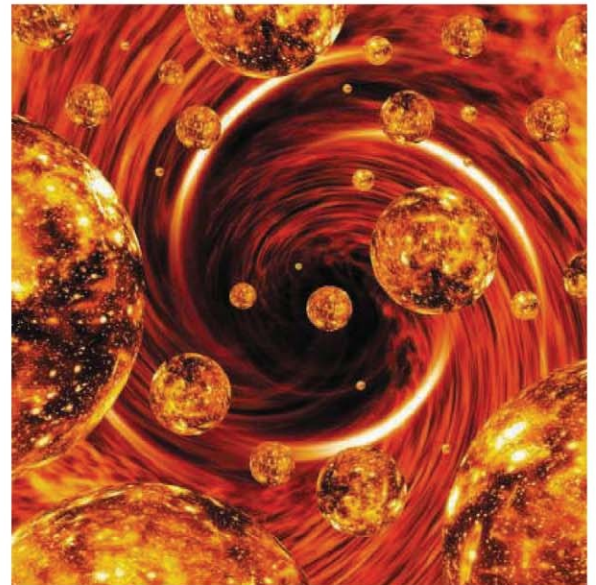
Nevertheless, M theorists manage to account for these dilemmas by combining them into the Goldilocks solution, more commonly called the anthropic principle. Our observed universe is not too hot, not too cold, but just right—or else we wouldn't be here to observe it. This solution satisfies many physicists but leaves many others implacably hostile to anthropic resolutions of physics problems. They prefer to wait for the emergence of a new physics theory of everything.

Once the extra dimensions of M theory have curled up, particle physics provides a plausible understanding of how the universe evolved. The fundamental forces of nature are unified at high energy. A phase of inflation (an immense expansion of space triggered in the earliest instants after the Big Bang) might account for the immense and nearly uniform universe we observe.

As Hawking points out, there is some evi-

dence that inflation actually occurred. Fluctuations in the cosmic microwave background radiation probe the earliest moments of the universe. Generic inflation predicted large temperature fluctuations, but we don't see these. They would conflict with the uniformity of large-scale structure, and we would be inundated with black holes. What inflation also predicts is the distribution of the fluctuations (as observed both in the microwave background and in large-scale galaxy surveys) and the flatness of space, so that the sum of the universe's kinetic and gravitational energy averages out to zero. The microwave background fluctuations indeed indicate that the average sum could be zero within the measurement uncertainties.

This observation leads Hawking to a remarkable prediction that will raise the pulsebeat of many readers. Here is his logic: M theory unifies gravity with quantum theory. Gravity accounts for negative energy, whereas the mass of a star is undeniably posi-



tive. On large enough scales, once one counts all the black holes, stars, and empty space, the overall energy of the universe is close to zero (as measured). If the universe has zero energy, then it could have been spontaneously created from nothing by quantum fluctuations. So for God, substitute M theory: "It is not necessary to invoke God to light the blue touch paper and set the universe going."

For Hawking, M theory is the only candidate for a complete theory of the universe. He argues that it is the most general supersymmetric theory of gravity, and that supersymmetry is obligatory to avoid the infinities that plague all previous attempts to develop a theory of quantum gravity. Some humbleness

The Grand Design

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and Leonard Mlodinow
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The reviewer, author of *Horizons of Cosmology: Exploring Worlds Seen and Unseen*, is at the Department of Astronomy, University of Oxford, Oxford OX1 3RH, UK, and the Department of Physics and Astronomy, Johns Hopkins University, Baltimore, MD 21218, USA. E-mail: j.silk1@physics.ox.ac.uk

would be welcome here. We are only a decade or two into M theory. A century or two hence, should we survive that long, I expect that M theory will seem as naïve to cosmologists of the future as we now find Pythagoras's cosmology of the harmony of the spheres, Ptolemy's cosmology of epicycles, or Kepler's cosmology of the five Platonic solids.

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PLANETARY SCIENCE

A Grand Tour in Its Historical Context

David J. Stevenson

Exploration has often been motivated by politics or by the closely related urge for financial gain. Visits to Earth's fellow planets were initially driven by the Cold War but have continued beyond that. We are currently in the golden age of exploration of our solar system. Although scientists might view this as a successful argument for the value of curiosity-driven science, fortunately for us the romantic urge and enthusiasm for the unknown also find support among the general public, the people who pay for it.

In the excellent and engaging *Voyager*, historian Stephen Pyne (Arizona State University) considers one aspect of planetary exploration, a highly successful mission whose two spacecraft visited the major planets (Voyager 2 flew by all four: Jupiter, Saturn, Uranus, and Neptune). In the book's subtitle, he not only recognizes the distinctive feature of Voy-

ager's goal (seeking newer worlds) but also places the endeavor in a historical context: the third great age of discovery. Pyne identifies the first such age with the great ocean-going voyages of exploration (think of Christopher Columbus or Ferdinand Magellan)

and the second with a lengthy period during which the emphasis shifted from loot to learning (think of James Cook and his scientifically motivated trip to Tahiti). He sees the third as having begun with the International Geophysical Year (1957–1958). The boundar-

ies between these ages may not be as sharp as Pyne posits, but that doesn't matter for the story he tells. Although he could have chosen Mars exploration instead of the Voyagers—for Mars captures the minds of the paying public (perhaps because it is more similar to Earth than are the other targets)—I believe that his choice was the best one: Voyager is a saga of enduring significance in human exploration. The scientific return was spectacular, the engineering challenges were great but overcome, and the story of Voyager lends itself to Pyne's historically based exposition. Importantly, he chose robotic exploration, not manned missions, for the use of astronauts is severely limiting, especially if one favors the return of important scientific information.

Voyager benefited from a fortunate near-alignment of the major planets at a time when the technology (scientific instruments and computers) was barely enough to do the job. The spacecraft were able to reach more distant bodies only through gravity assist, which was based on physics known long before the mission but required careful computation to convert theory into practical trajectory design. Even though Pyne describes all this and much more, a considerable part of the book's strength lies in the fascinating excursions into the analogies with the first and second great ages of exploration. He weaves these into his roughly chronological story of Voyager so well that they do not seem like digressions and instead are more of an explanatory basis for the human restlessness that drives our urge to explore. In all ages, there are common techniques (as in the use of stars for navigation), common intrigues (e.g., political and funding difficulties and clashes of personalities), and common motivations. The costs of missions are forever underestimated—just as Queen Isabella's treasury underestimated the costs of the Columbus voyages.

The book pays relatively little attention to the importance of the scientific returns from the two spacecraft and the story of how Voyager (and subsequent missions, especially Galileo and Cassini) has changed our view of our solar system, how it formed and evolved, and how it works. Pyne, wisely, does not attempt to cover all of this, and a book that does has yet to be written.

Pyne mentions "Ernest Shackleton's celebrated advertisement in the *London Times* ...: 'Men wanted for hazardous journey. Low wages, bitter cold, long hours of complete



Colored clouds of Jupiter. Voyager 1 took this image of the Great Red Spot during its 5 March 1979 encounter with the planet.

darkness. Safe return doubtful. Honour and recognition in event of success'" (*1*). Modern planetary scientists and engineers suffer less privation, but success can still be elusive. As in space missions, Shackleton had to decide which data to lose: the photographic plates destroyed when the expedition abandoned its ice-bound ship because they weighed too much. Today's robotic undertakings lack the visceral quality of the old style of manned exploration, and future endeavors will look more and more like virtual experiences (video games). In the United States, NASA's annual budget for new planetary missions is less than the amount consumers spend on video games in a month. Perhaps the fourth great age of exploration will be the return of (and visualization of) data from planets around other stars—something unlikely to be accomplished by sending spacecraft to their vicinity but possibly within the power of clever telescopes. Still, the utility of spacecraft visits to distant places is far from exhausted, and the Voyagers are still calling home and informing us about the boundary between our solar system and interstellar space.

The book's appendix offers a helpful chronology of successful lunar and planetary missions as well as useful diagrams and tables. Unfortunately, the 11 photographs included in the book (as black-and-white prints) are rather standard and inferior to those you can view on your own computer from NASA sites. (One wonders why editors choose to reproduce inferior photos.) Nonetheless, I heartily recommend Pyne's *Voyager* for its scholarly yet lively story.

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2. www.antarctic-circle.org/advert.htm.

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Voyager
Seeking Newer Worlds
in the Third Great Age
of Discovery

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The reviewer is in the Division of Geology and Planetary Sciences, California Institute of Technology, Pasadena, CA 91125–2100, USA. E-mail: djs@gps.caltech.edu

SCIENCE AND REGULATION

Regulating Direct-to-Consumer Personal Genome Testing

Amy L. McGuire,^{1*} Barbara J. Evans,² Timothy Caulfield,³ Wylie Burke⁴

Direct-to-consumer (DTC) personal genome tests claim to provide consumers access to information about their genetic ancestry, susceptibility to traits such as excessive earwax, carrier status for diseases like cystic fibrosis, ability to metabolize drugs like statins, and likelihood of developing diseases such as cancer, Alzheimer's disease, and diabetes—all in one test, for a few hundred dollars, and without involvement of a health-care professional. Proponents of such tests tout the power of making such information easily available, while critics worry about consumer safety and harm that could result from unreliable tests, excessive claims about the meaning of tests or the benefits of being tested, and misinterpretation of test results (1, 2). The U.S. Government Accountability Office (GAO) raised concerns (3, 4), and the U.S. Federal Trade Commission (FTC) warned consumers to interpret at-home genetic tests with “a healthy dose of skepticism” (5). In 2009, the House Energy and Commerce Committee initiated an investigation into regulation of DTC testing (6).

Many problems of DTC tests reflect problems of genetic tests more generally. Well over 90% of genetic tests available in the United States do not receive data-driven review by an external regulator to confirm safety and effectiveness before moving into clinical use (1). There is broad consensus about the need for some form of regulatory review before allowing use of a genetic test (7), but there has been ongoing controversy about how to achieve this goal. Effective regulation will require cooperation from governmental agencies, and flexibility to accommodate the complexities of tests, like those offered by DTC companies, that provide genome-wide analysis producing results with variable and often uncertain validity and clinical utility.

U.S. Federal Regulation

Laboratories that provide clinical testing services in the United States—such as diagnostic or genetic testing—are regulated under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) (8). The CLIA regulations address the quality of lab testing services, for example, by ensuring that laboratories are properly staffed and follow proper procedures. The genetic test kits that laboratories purchase from medical device manufacturers receive additional regulation by the U.S. Food and Drug Administration (FDA) as in vitro diagnostic devices (9). However, if a lab develops a test in-house [lab-developed test (LDT)], as opposed to purchasing the test from a device manufacturer, the test may escape FDA oversight. A lab cannot sell its LDTs for use by other laboratories but can use them itself to provide testing services to the public. Many LDTs arguably fall within the definition of a “device” that is subject to FDA regulation, but FDA, in an exercise of its enforcement discretion, has traditionally held back from regulating most LDTs (10, 11).

This policy has a profound impact on the level of evidence required before a test can move to market. Lab-developed tests receive internal validation at the lab that developed them but do not generally receive data-driven review by a regulator to ensure their safety and effectiveness, nor are they subject to the postmarket vigilance and adverse event reporting that FDA regulations provide.

In contrast, FDA-regulated tests receive data-driven review. The precise data requirements depend on how the test is classified in terms of novelty and level of risk, based on factors such as the manufacturer's clinical claims (e.g., does the test predict cancer susceptibility or merely earwax type?) and the seriousness of interventions that might be taken in response to the test results (e.g., prophylactic mastectomy). Higher-risk genetic tests may be required to move through FDA's premarket approval (PMA) process, which requires at least some clinical trial evidence to show safety and effectiveness before introducing the product in the market. Moderate-risk tests would pass through the less-rigorous 510(k)

International cooperation and postmarket regulation are needed for Internet-based direct-to-consumer genome tests.

clearance process. The 510(k) clearance process does not necessarily require clinical trials but does require premarket research to support the device's risk classification and to validate any analytical or clinical claims that the sponsor plans to make about the device. Either way, some data-driven external regulatory review is required before a test can be sold for commercial use.

Regulating DTC Tests

DTC genetic tests may escape premarket review by FDA under a business model in which consumers send their samples to a CLIA-certified lab that performs testing using its own LDTs. In response to this concern, FDA recently sent letters to multiple companies involved in DTC testing (12), signaling its intent to assert jurisdiction over

No one regulatory strategy will be suitable for all DTC tests.

DTC genetic tests. In July 2010, the FDA sponsored a public meeting to gather input on appropriate regulation of all LDTs, including DTC tests (13).

There are two key barriers to FDA oversight of DTC tests: (i) there is a lack of data to support premarket clearance or approval, and (ii) even when data support the test's intended use, FDA cannot control off-label use (use in ways other than those intended by the manufacturer or reviewed by regulators). Although there has been speculation about the potential psychosocial harms of testing, such as an increase in anxiety or encouragement of fatalistic behavior, there are, to date, few studies addressing these concerns (14). The limited evidence tends to be reassuring, even for risk information associated with relatively serious ailments (15, 16). However, iatrogenic harm (harm due to treatment) may be a more serious concern. In one study, 40% of participants with genetic test results indicating increased risk for Alzheimer's disease reported increasing their use of medications or vitamins, compared with 20% of those whose results did not indicate increased risk (16). Given that all medications carry the potential for adverse effects, the scope of

¹Center for Medical Ethics and Health Policy, Baylor College of Medicine, Houston, TX 77030, USA. ²Health Law and Policy Institute, University of Houston Law Center, Houston, TX 77204, USA. ³Health Law Institute, University of Alberta, Edmonton, AL T6G 2H5, Canada. ⁴Department of Bioethics and Humanities, University of Washington School of Medicine, Seattle, WA 98195, USA.

*Author for correspondence. E-mail: amcguire@bcm.edu

potential harm from unnecessary or unproven treatment after genetic risk assessment is an important unstudied question.

There is a need for data, but there are genuine economic and feasibility issues in generating it before tests go on the market (11). Many personal genome tests provide predictive information, with the ultimate goal of long-term disease prevention. Thus, there may be decades of uncertainty before the risks and benefits are truly understood. Delaying consumer access to DTC tests until robust pre-market data are available would inhibit product development and could effectively regulate DTC companies out of existence.

The solution lies in accepting that pre-market studies cannot fully answer questions about the safety and effectiveness of genetic tests and focusing additional effort on post-market surveillance and regulation. This mirrors the approach taken for drugs in the FDA Amendments Act of 2007 (FDAAA) (17), which expands FDA's authority to require ongoing studies after drugs are approved and calls for creation of a large data network to support outcomes-based observational studies (11). It would probably require new legislation to give FDA a complete set of FDAAA-like powers to regulate devices.

Moving Forward

No one regulatory strategy will be suitable for all DTC tests. Even within a single DTC test, multiple risk assessments are provided and each must be evaluated independently. We therefore support the recommendation for a risk-stratified approach (1). FDA premarket review would focus on higher-risk tests. Less-risky tests that receive lighter FDA premarket scrutiny would still be subject to other oversight mechanisms, such as a national registry or measures to disclose uncertainties and risks. Risk-stratification criteria have not yet been developed and remain an important policy priority.

All tests should be analytically valid (able to accurately and reliably measure what they say they are measuring), and any clinical claims made about the test must be accurate and substantiated. Safety and effectiveness data should be developed, but for many tests, this should be done through enhanced postmarket surveillance and clinical studies, rather than a more stringent premarket approval process. Premarket assessment would focus on identifying tests with potential for egregious harms (e.g., tests with uncertain validity or utility that could profoundly alter the course of medical treatment) and keeping those tests off the market until further studies show an acceptable risk-benefit ratio.

Finally, tests that fall within the highest risk classification should only be performed with pre- and/or post-test counseling by a licensed health care professional. There is general agreement among most professional organizations that genetic testing should only be offered under the supervision of a qualified health professional (5, 18, 19). However, mandatory physician involvement for all tests would limit access to tests that ultimately prove low risk and, without clear professional guidelines and education, could exacerbate the problem of unnecessary and potentially harmful and expensive follow-up based on test results of unproven clinical significance (20).

For clinicians—and anyone contemplating such testing—the key to responsible and safe use will be access to unbiased information. In the United States, both FDA and FTC could help ensure more accurate and scientifically informed testing claims and frank disclosure of what is still not known about a test. FTC and FDA share jurisdiction over regulating deceptive advertising, but FTC has responded to concerns primarily through public education, reserving its policing authority for cases of egregious abuse (21).

More informal policy action should also play a role. The proposed National Institutes of Health (NIH) Genetic Testing Registry (22) should be used to ensure more complete and systematic public disclosure of testing information by test developers. Publicly funded resources that provide authoritative information about clinical utility—such as GeneTests (23) and the Genomic Applications in Practice and Prevention (GAPP) Knowledge Base (24)—should be expanded.

Of course, the United States is not the only country to have considered the regulatory challenges associated with DTC genetic services (25). Germany, for example, has taken aggressive steps, banning public access to private tests (26). The response in the United Kingdom has primarily been through the production of policy reports (27). Given that this is largely an Internet-based industry, a comprehensive regulatory policy will need to consider international laws, local norms, and implications for stakeholders from diverse health systems. International cooperation will be required.

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MOLECULAR BIOLOGY

Surfing Chromosomes (and Survivin)

Andrea Musacchio

Mothers are generally inclined to provide each of their offspring with similar opportunities. Even a mother cell abides by providing her daughters with an equal dowry of chromosomes. Diversions from fairness create imbalances in chromosome numbers that affect cell viability and are associated with tumor formation, infertility, and birth defects. Three papers in this issue—by Yamagishi *et al.* on page 239 (1), Wang *et al.* on page 231 (2), and Kelly *et al.* on page 235 (3)—describe how an enzyme (Aurora B protein kinase) that is essential for accurate chromosome inheritance is recruited to chromosomes that are poised to segregate into daughter cells.

Chromosomes attach to the microtubules of a spindle apparatus that separates them during cell division (mitosis), a process that is error prone. By reversibly modifying target proteins with a phosphate group on serine or threonine residues, Aurora B acts as a fidelity factor (so-called checkpoint) for this process. Inhibiting Aurora B leads to errors in chromosome attachment to the spindle, and to premature exit from mitosis, which is indicative of checkpoint failure (4).

Current models posit that a centromeric pool of Aurora B corrects attachment errors. A centromere is a chromosomal region that

specifies the formation of a kinetochore, a complex protein assembly devoted to spindle capture (see the figure). Phosphorylation of substrates at the centromere and kinetochore by Aurora B may control the “fluidity” of the kinetochore-spindle interface, preventing premature stabilization of the interaction. This would allow resolution of improperly formed connections.

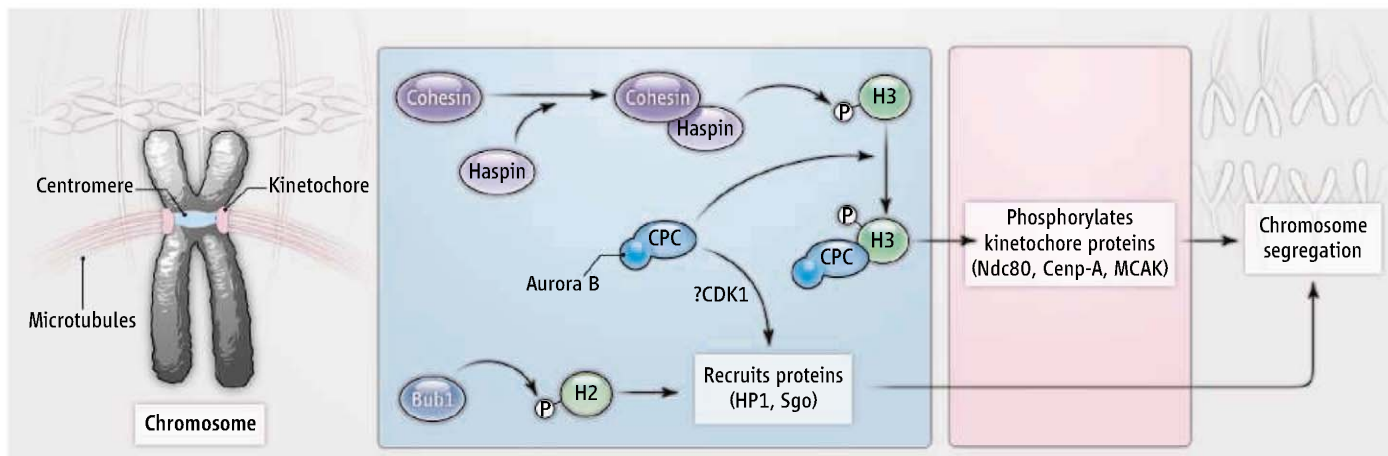
The signals that attract Aurora B to the centromere had been unknown. Now, Yamagishi *et al.*, Wang *et al.*, and Kelly *et al.* report that a conserved protein kinase, Haspin, is crucial for this process in three distinct model organisms, including humans. Haspin is an atypical protein kinase that phosphorylates threonine 3 of histone H3 (P-Thr³-H3). Kelly *et al.* show that Survivin, a protein that binds to Aurora B in the chromosome passenger complex (CPC), contains a bipartite binding site that recognizes the phosphate moiety of P-Thr³-H3 and also an alanine residue at the amino terminus of histone H3. Thus, the CPC may be recruited to mitotic centromeres by interacting with P-Thr³-H3. But what determines the localization of this modification mark specifically at centromeres? Yamagishi *et al.* observed that Haspin interacts with Cohesin, a protein complex required for sister chromatid cohesion. Because the bulk of Cohesin resides at centromeres during mitosis, P-Thr³-H3 may be generated at these chromosomal regions through the interaction of Haspin with Cohesin.

An enzyme that localizes to mitotic chromosomes phosphorylates histones, leading to the recruitment of proteins that ensure proper chromosome segregation.

An unresolved puzzle concerns the mechanisms through which Aurora B gains access to its substrates. Specifically, Aurora B targets proteins at the interface of the centromere and the inner kinetochore, including Cenp-A, mitotic centromere-associated kinesin (MCAK), Ndc80, and Knl1 (4). The ability of Aurora B to target kinetochore substrates is inversely proportional to the degree of tension impinging on kinetochores (5, 6). Kinetochore tension arises from the binding of microtubules. Tubulin polymers are the main constituent of the mitotic spindle and are important for discriminating correct from incorrect kinetochore-spindle attachments (7). Tension stretches kinetochores, so that certain kinetochore constituents become separated by ~30 to 50 nm (8). As the kinetochore stretches, kinetochore substrates may be put beyond the reach of Aurora B that is localized at the centromere, leading to the stabilization of attachment and to checkpoint satisfaction. It is unclear how kinetochore tension causes a progressive separation of centromeric Aurora B from its kinetochore substrates (8).

Yet, the effects of tampering with Haspin-mediated localization of Aurora B to the centromere are not as pervasive as one might expect. Wang *et al.* show that at the interface between the centromere and inner kinetochore, the extent of phosphorylation of Cenp-A was not appreciably affected by depletion of P-Thr³-H3 in mammalian cells. By contrast, the recruitment of MCAK to centro-

Department of Experimental Oncology, European Institute of Oncology, Via Adamello 16, I-20139 Milan, Italy. E-mail: andrea.musacchio@ifom-ieo-campus.it



Kinase recruitment. A cascade of molecular interactions and phosphorylation (P) events occur at the centromere and inner kinetochore of sister chromatids that are attached to the mitotic spindle (microtubules), poised to segregate.

These include recruitment of the kinases Haspin and Bub1, as well as the CPC complex (which harbors the Aurora B kinase). These events control the normal segregation of chromosomes during cell division.

meres and kinetochores, which depends on phosphorylation by Aurora B, was blocked. Cenp-A and MCAK are considered bona fide substrates of Aurora B. The strong difference in Cenp-A and MCAK phosphorylation upon Aurora B mislocalization is surprising. Perhaps the degree of substrate phosphorylation is determined by selective depletion or retention of specific pools of Aurora B. For example, there may be a diffusible pool of the kinase that does not require centromere recruitment to phosphorylate Cenp-A. Alternatively, there may be specific centromeric pools of Aurora B, each recruited through a distinct modification to distinct targets.

Through studies in human and fission yeast cells, Yamagishi *et al.* have implicated a second mark, phosphorylated threonine 120 of histone H2A (P-Thr¹²⁰-H2), in the recruitment of the CPC to centromeres (9, 10). This mark, created by the protein kinase Bub1, is proposed to recruit Shugoshin proteins (hSgo1 and hSgo2 in humans). These, in turn, may recruit components of the CPC (Survivin or Borealin), provided that they have

been previously phosphorylated by cyclin-dependent kinase 1 (CDK1) (10). Bub1 and Sgo2, like Aurora B, are required for MCAK recruitment to the centromere and inner kinetochore in human cells (11), in agreement with the hypothesis that these proteins control Aurora B localization and thus MCAK recruitment. However, there are contradictory reports in the role of Bub1 in Aurora B localization in human cells (12, 13). Furthermore, previous studies showed that Aurora B acts upstream, not downstream, of Shugoshin proteins in human cells (11, 14). Thus, further clarity is needed on the role of Bub1 and Shugoshin in Aurora B recruitment.

There likely are additional needles in the CPC protein modification haystack. Dephosphorylation of Incenp (Sli15p in *Saccharomyces cerevisiae*), another component of the CPC, is crucial for removing the CPC from the centromere and relocating it to the spindle after chromosomes have segregated to opposite poles of the dividing cell (15), suggesting yet additional mechanisms for centromere recruitment or retention of the CPC.

Experiments centered on reconstituting the CPC and its interactions may be ultimately necessary to illuminate the path and make sense of this complexity.

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PHYSICS

Looking at How Things Slip

Stefano Zapperi

To displace a body resting on a surface, a lateral force that is larger than the static friction force must be applied. The ratio of the friction force and the normal force (the coefficient of static friction) depends on some properties of the contact interface, such as the material type and the surface roughness, but not on the apparent contact area. These macroscopic laws of friction, formulated by Amontons at the end of the 17th century, remain under investigation today (1). An understanding of how these macroscopic forces originate in microscopic processes has application in materials and engineering and may even improve the modeling of earthquakes. On page 211 of this issue, Ben-David *et al.* (2) report careful measurements of local shear and normal stresses across a contact interface during

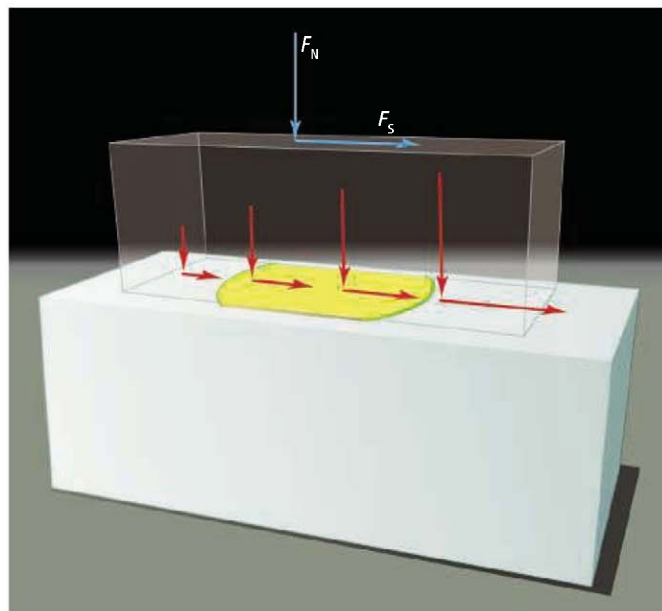
frictional slip that show considerable non-uniformity. Although Amontons' laws apply globally to the entire system, they do not hold locally. The results of the study challenge the commonly held assumption that the lat-

New measurement techniques are overturning some long-held assumptions related to the microscopic processes controlling friction.

eral and normal forces are uniform across the contact interface and are thus likely to change the way we model friction.

Ben-David *et al.* use optical methods to monitor the area of real contact between two blocks made of a transparent polymeric material (see the figure). The onset of friction occurs by the nucleation and propagation of a detachment front breaking the contact interface, in a way that resembles the dynamics of a shear crack (3). They find that the speed of frictional fronts increases with the

Slipping away. In a friction experiment, two blocks in contact are subject to uniform normal (F_N) and lateral (shear) forces (F_S), depicted as blue arrows. On the contact interface, however, Ben-David *et al.* (2) show that normal and shear stresses (red arrows) are not uniform. Slip occurs by the nucleation of a detachment region (yellow) and by the propagation of detachment fronts (green). The front velocity depends on the local ratio between shear and normal stresses.



CNR-IENI, Via R. Cozzi 53, 20125 Milano, Italy, and ISI Foundation, Viale San Severo 65, 10133 Torino, Italy. E-mail: stefano.zapperi@cnr.it

ratio between shear and normal stresses, $\tau(x)/\sigma(x)$. They identify three dynamical regimes for the front velocity that are in principle observable in earthquakes; hence, they suggest that knowing the state of stress in a fault could help to predict the kind of earthquake to be expected. Similar considerations could also apply to landslides or snow avalanches. However, further work would be needed to move from the ideal conditions of the present experiment to more realistic ones that would be relevant for geophysical phenomena.

Apart from the possible impact for Earth sciences, these experimental results pose challenging problems to theorists. We would like to understand how heterogeneous stress fields on the contact interface can influence the nucleation and propagation of detachment fronts. Ben-David *et al.* note that slip nucleation typically occurs in regions where $\tau(x)/\sigma(x)$ is large, but a more quantitative understanding of this process is lacking. A possible path to clarify this issue could be based on analogies with other phenomena that are ruled by the nucleation and propagation of fronts moving in a heterogeneous landscape. For example, magnetization reversal under an external magnetic field, as manifest in ferro-

magnetic hysteresis, occurs by the nucleation of domains and the subsequent motion of domain walls (4). It was recently shown (5) that the onset of slip for two perfectly ordered atomic-scale surfaces can be described by the classical theory of nucleation. Using this theory, the energy barrier for nucleation can be estimated by comparing the energy gain associated with the slip with the energy cost needed to create a detachment front. A promising approach to generalizing this to heterogeneous stress profiles across the contact interface is provided by mesoscopic friction models, in which the elastic body is considered to be a set of discrete blocks connected by springs (6).

Another possible path to further progress derives from the similarity between the experimental diagram relating the front velocity to $\tau(x)/\sigma(x)$ and the depinning transition of elastic interfaces in disordered media (7), a well-studied problem relevant to a wide variety of phenomena including the motion of ferromagnetic or ferroelectric domain walls, the spreading of liquid drops on surfaces, and many others. In all of these cases, the velocity of the front increases with the driving force; for low values of the driving force, the inter-

face would be fixed (or pinned), but thermal fluctuations would lead to slow creep motion. When the force is larger than a critical threshold, the front moves with a velocity that scales with the difference between the driving force and the threshold force (depinning). It is tempting to describe the onset of frictional slip in terms of the depinning of a detachment front driven by $\tau(x)/\sigma(x)$ with a pinning threshold set by the coefficient of static friction. Whether this analogy can lead to a better quantitative understanding of friction still remains to be investigated.

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PLANT SCIENCE

Saving the Bilayer

John Browse

During the cool, shortening days of fall, plants of the high latitudes prepare for winter survival through a process of cold acclimation. For the model plant *Arabidopsis thaliana*, cold acclimation improves freezing tolerance from -5°C to -12°C . Other plant species can survive considerably lower temperatures (1), but it is believed that the underlying biochemical processes are similar. This means that discoveries made in *Arabidopsis* are generally valuable for understanding and improving the freezing tolerance of plants. On page 226 of this issue (2), Moellerling *et al.* provide a satisfying explanation for an enzyme and a process that are absolutely essential for plants to survive freezing.

During freezing, ice first forms in the extracellular space, causing loss of water from the plant cell by osmosis. Thus, changes in gene expression that occur during cold acclimation and freezing overlap substan-

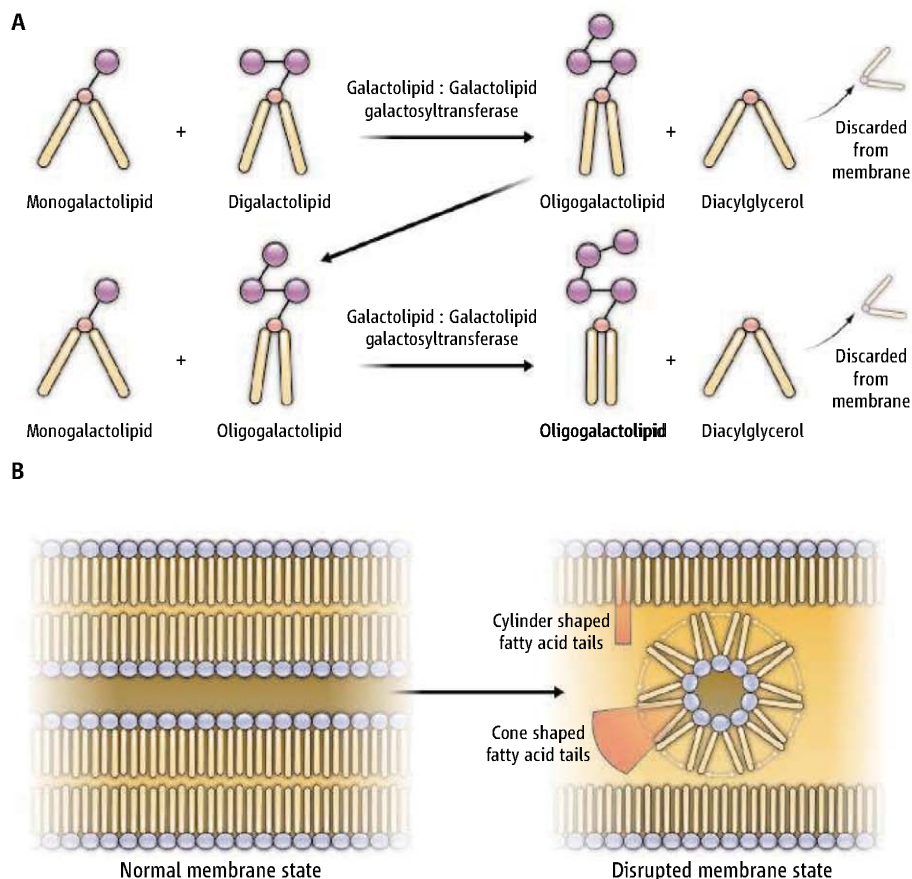
tially with those reported for responses to dehydration and salt stress in plants (3). Screens of *Arabidopsis* for induced gene expression have identified transcription factors—most importantly five CBF/DREB proteins—whose activities increase tolerance to freezing temperatures (3, 4), as well as other components of the cellular signaling pathway of cold acclimation (5). Analyses of gene, protein, and metabolite expression in mutant plants deficient in cold-stress signaling, or that overexpress CBF/DREB transcription factors, revealed extensive genetic and metabolic changes during cold acclimation. For example, at least 1000 *Arabidopsis* genes are altered in expression (5).

It is evident that freezing tolerance is an induced state, with substantial costs to normal metabolism and growth. However, the transcriptional responses observed do not preclude the possibility of changes involving constitutively expressed factors whose activities may be altered at the biochemical level. Two screens with the potential to uncover such

Membrane lipids must be metabolized to protect plants from frost.

constitutively expressed components were designed to identify *Arabidopsis* mutants that can survive freezing conditions without having first acclimated to cold temperatures (6) or that remain sensitive to freezing even after cold acclimation (7). The latter screen identified mutations in the gene *sensitive to freezing 2* (*sf2*) that have no discernible effect on growth or development of plants at temperatures as low as 4°C . However, mutant plants die following cold acclimation and exposure to -6°C for 24 hours, even though cold acclimation appears normal (7, 8). SFR2 was identified as a member of the glycosyl hydrolase family 1 enzymes. Recombinant SFR2 showed glycosyl hydrolase activity, but so did SFR2(G234D), the variant encoded by the *sf2-1* mutant allele. The original analysis of the SFR2 protein identified a putative signal sequence that suggested it might be secreted from the cell (9). However, more-reliable evidence eventually placed SFR2 in the chloroplast envelope, and examination of leaf tissue by electron microscopy suggest that the chlo-

Institute of Biological Chemistry, Washington State University, Pullman, WA 99164, USA. E-mail: jab@wsu.edu



Lipid conversion. (A) In the plant chloroplast outer membrane, the galactolipid:galactolipid galactosyltransferase (GGGT) encoded by *SFR2* converts cone-shaped monogalactolipids to cylindrical oligogalactolipids and diacylglycerol (which is removed from the bilayer). (B) A series of nonbilayer H_{II} structures can form from adjacent leaflets of closely appressed bilayers. It is proposed that GGGT prevents this by removing the H_{II} -forming monogalactolipids.

roplasts are the initial site of damage after freezing in *sf2* mutant plants (10).

To determine how mutations in *sf2* leave plants susceptible to freezing, Moellering *et al.* considered the function of *SFR2* as a glycosyl transferase instead of a hydrolase, a known alternative activity of this class of proteins. Galactolipid:galactolipid galactosyltransferase (GGGT) is an enzyme in the chloroplast outer envelope that converts monogalactosyldiacylglycerol (MGDG) to oligogalactosyldiacylglycerols, with the concomitant production of diacylglycerol (see the figure) (11). Lipid analyses of leaf tissue indicated that *sf2* mutants are indeed deficient in GGGT activity, and a membrane fraction from yeast cells expressing *Arabidopsis SFR2* exhibits GGGT activity against MGDG. On this evidence, it is easy to accept that the GGGT activity of *SFR2* makes the difference between life and death in the wild type and in *sf2* mutant plants.

Moellering *et al.* propose two possible hypotheses for the essential role of *SFR2* during freezing. Because it has a small head

group (a single galactose residue), MGDG has a propensity to form a nonbilayer hexagonal II (H_{II}) structure (see the figure). This structure can form from closely appressed lipid bilayers and disrupt the biochemical activities and barrier function of the membranes. H_{II} structures were proposed to cause fusion between the chloroplast outer envelope and the plasma membrane during freezing of *Arabidopsis* protoplasts isolated from tissue that was not cold-acclimated (12). Converting MGDG to oligogalactolipids is expected to reduce the propensity for H_{II} formation between tightly appressed membranes during freezing-induced dehydration. In addition, the increased size of the head group domain of oligogalactolipids and the increased density of hydroxyl groups (and therefore, charge) per unit of membrane surface area may enhance repulsive forces between appressed membranes. The second potential mechanism centers on the net removal of membrane glycerolipids that occurs by the action of GGGT, coupled with conversion of diacylglycerol to triacylglycerol. Moellering *et al.* propose that

this removal reduces the total area of the chloroplast membrane and thereby accommodates shrinkage of the organelle due to osmotic loss of water during freezing. However, in a different context, such loss of surface area has been proposed to cause lysis during the osmotic expansion that occurs upon thawing (13).

How quickly can *SFR2* change membrane composition at subzero temperatures? Moellering *et al.* found that in wild-type plants there was no lipid change after cold acclimation, and their measurements on freeze-treated plants were made after 36 to 48 hours of freezing. This is considerably longer than a typical frosty night. In this context, it would be useful to demonstrate [by freeze-fracture EM (12) or other techniques] that H_{II} formation occurs in cold acclimated *sf2* mutant plants. At normal temperatures, the major glycerolipid in the outer leaflet of the chloroplast outer envelope is phosphatidylcholine, and only a small proportion of MGDG is present in that leaflet (14, 15). Do these proportions change during cold acclimation? Perhaps GGGT, coupled with lipid transfer alters MGDG content of other chloroplast membranes that might otherwise be involved in fatal H_{II} formation. Now that the cryptic GGGT enzyme has been assigned a definitive role in protecting plants from freezing, these additional questions can be addressed.

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MICROBIOLOGY

Interacting Parasites

Kevin D. Lafferty

Parasitism is the most popular life-style on Earth, and many vertebrates host more than one kind of parasite at a time. A common assumption is that parasite species rarely interact, because they often exploit different tissues in a host, and this use of discrete resources limits competition (1). On page 243 of this issue, however, Telfer *et al.* (2) provide a convincing case of a highly interactive parasite community in voles, and show how infection with one parasite can affect susceptibility to others. If some human parasites are equally interactive, our current, disease-by-disease approach to modeling and treating infectious diseases is inadequate (3).

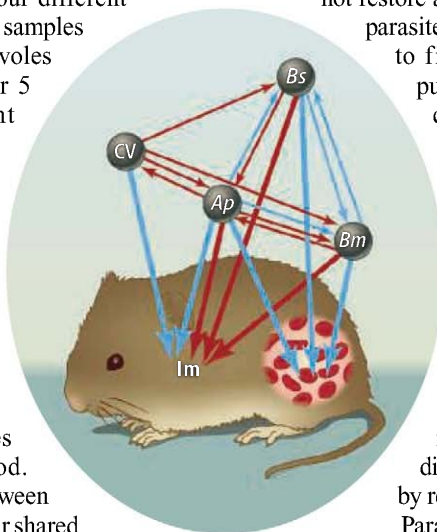
Telfer *et al.*'s study—which involved tracking infections of four different parasites by taking blood samples from nearly 6000 wild voles (*Microtus agrestis*) over 5 years—helps highlight our growing understanding of how parasites can interact in complex ways (see the figure). What are some of the take-home messages?

Parasites are consumers and can compete for resources. In Telfer *et al.*'s voles, for instance, some parasites may compete for blood. Because competition between parasites increases as their shared resource becomes limited (4), parasites that grow or reproduce substantially within the host are more likely to compete (5). Early experiments demonstrated that one kind of intestinal parasite, acanthocephalan worms, displaced tapeworms from the best sites within the intestine and competed for food (6). Studies have also indicated that the malaria parasite competes with parasitic worms for red blood cells, a finding with important implications for human health (7).

Parasites also apparently engage in competition through a phenomenon called cross immunity (3). Immune system cells, such as memory T cells, produced in response to one

parasite can cross-react with antigens from similar parasite species (8). For this reason, infection with one species of human schistosome (a trematode worm) can protect against new infections by other schistosome species (9). Cross immunity to a wider range of parasites can arise after the immune system's generation of a network of regulatory cells and cytokines in response to infection (8). In Telfer *et al.*'s study, cross immunity could explain why voles infected by the protozoan *Babesia microti* show reduced susceptibility to *Bartonella* bacteria, but the result also could indicate competition for blood cells. Whatever the mechanism, targeting treatment of one parasite in a mixed infection might

not restore a patient to health if the parasite's competitor responds to fill the void. Similarly, public health campaigns could have net negative



Vole parasite interactions. Four pathogens—cowpox virus (CV), the protozoan *B. microti* (Bm), and two bacteria, *A. phagocytophilum* (Ap) and *Bartonella* spp. (Bs)—can have positive effects (red lines) and negative effects (blue lines) on each other (2). Thick lines are proposed direct effects on the host vole (*M. agrestis*) and thin lines are proposed indirect effects among parasites. Im represents the immune system, and the inset circle represents a limited pool of red blood cells.

consequences if they inadvertently promote disease-causing parasites by removing competitors.

Parasites sometimes facilitate each other. Co-infections with dissimilar parasites can spread the immune system thin (3). Shedding of the severe acute respiratory syndrome (SARS) virus increases, for instance, if a person has a concurrent pulmonary infection; for this reason, a few co-infected persons became super spreaders in the SARS epidemic (10). Parasites can also suppress the immune system, opening the door for others. Most notably, infection with HIV facilitates opportunistic bacterial, fungal, protozoal, and viral pathogens (11). Indeed, HIV's ability to suppress the immune system is the principal cause of its devastating morbidity and mortality in untreated cases. As Telfer *et al.* mention, HIV also increases the potential for tuberculosis to spread to the general population (12). Parasitic worms can also suppress the inflamma-

Parasites interact in complex ways in the voles they infect.

tion response of the immune system, making it easier for certain protozoan parasites to succeed (7). In voles, cowpox appears to temporarily impair the immune system and increase their susceptibility to other parasites (2). If it is easier to control a facilitating parasite than a disease agent it facilitates, then targeting the facilitator could be an efficient way to manage epidemics.

When do interactions matter? The answer can depend on the strength of the interactions, the prevalence of potential interacting species, and various factors that tend to intensify interactions or isolate species from one another in space and time (13). The finding that infection with one parasite greatly increases susceptibility to infection by a second parasite is meaningful only to the extent that the host is exposed to both parasites in nature. Voles, for instance, are much less frequently exposed to *Anaplasma phagocytophilum* bacteria than

they are to other parasites, reducing the influence of this particular parasite on community dynamics, despite its potentially strong effects on the other species. To isolate how one parasite affected susceptibility to other parasites, Telfer *et al.* used statistical techniques to control for confounding factors. This was essential to quantify per-capita susceptibility, but the approach also obscured factors that might affect the frequency of interactions at the host population level. Now that they have illustrated the strength of interactions, Telfer *et al.* have the opportunity to consider whether environmental, spatial, temporal, or demographic factors increase or decrease the frequency of coexposure to parasites.

Voles have more to worry about than the network of four pathogens studied by Telfer *et al.* What would infection patterns look like if the several parasitic worms that infect voles (14) were included? Worms can interact strongly with viruses, bacteria, and protozoa (7). How might immune-modulated effects and competition for resources interact? In

Western Ecological Research Center, U.S. Geological Survey, c/o Marine Science Institute, University of California, Santa Barbara, CA 93106, USA. E-mail: kevin.lafferty@lifesci.ucsb.edu

natural ecosystems, strong predation pressure can reduce the abundance of competitors so that resources are no longer limiting (15). This basic premise of community ecology should apply to parasite communities as well, where the host immune system can act as a predator on parasites. An impaired immune system (like release from predation in free-living communities) should set the stage for more intense competition among parasites. This is a key difference between free-living systems and parasite communities, because prey are less likely to impair predator populations. Even more challenging to predict are the myriad indirect interactions within a community of parasites. If parasites can affect each other indirectly through long causal chains, the study of parasite communities could benefit from modern approaches to dealing with complexity, such as network theory and structural equation modeling.

A further challenge for parasite ecologists will be to examine how effects on host susceptibility translate into effects on host and parasite population dynamics (3). In addition to affecting susceptibility, parasites can interact through their negative effects on host survivorship and population densities, leading to the potential for complex feedbacks among pathogens at the population level. In addition, interactions among parasites, and between parasites and the immune system, have the potential to alter the course of virulence evolution in parasites (16).

There is enough evidence that human parasites interact to motivate systematic investigations of parasite communities in human populations. Telfer *et al.* provide an example of how, through collecting infection data over time, one could quantify the importance of parasite interactions in humans. With such information, we would know better when

to stop treating and managing parasites one species at a time.

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CHEMISTRY

Inorganic Nanoparticles as Protein Mimics

Nicholas A. Kotov

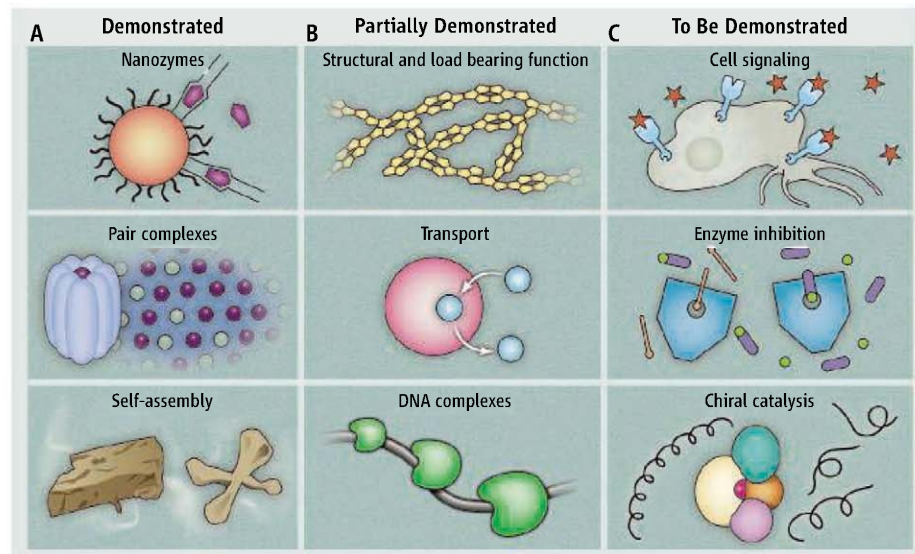
Water-soluble inorganic nanoparticles (NPs) and globular proteins (GPs) might seem “as different as chalk and cheese,” especially in the interior. The chemical structure of GPs is usually exact and well-defined, whereas NPs are almost always formed as a mixture of sizes and variation of shapes. The complexity and dynamism of three-dimensional atomic organization inside the protein globules and related functionalities are not present in the impenetrable crystalline cores of NPs. However, NPs and GPs do reveal similarities in overall size, charge, and shape, and the exterior surfaces of NPs can be coated with organic functional groups similar to those exposed by GPs, which suggest that NPs could function as protein mimics. This option is attractive because NPs are usually cheaper and more stable than proteins, but can they actually display the same functionalities and achieve enough specificity to replace proteins?

The majority of preparation schemes of water-soluble NPs use thin coatings of small organic molecules, or stabilizers, with a vari-

ety of functional groups and some degree of anisotropy (1). The methods for separating, purifying, and solubilizing NPs and GPs are similar (2–4). Typical sizes of NPs and GPs are comparable to nanometer-scale features of cellular membranes, such as ion channels (5). The interactions of water-soluble NPs and

Inorganic nanoparticles coated with organic films can display surface chemistries that allow them to function like globular proteins.

GPs with the environment and other soluble molecules are virtually identical and depend on the same media parameters. For example, surface charges of both NPs and GPs depend on pH and ionic strength and can influence their binding interactions to cellular membranes (5). If needed, the NP coatings may also



Nanoparticles vie for protein jobs. Examples of (A) demonstrated, (B) partially demonstrated, and (C) potential functional similarities between water-soluble nanoparticles and globular proteins.

Department of Chemical Engineering, Department of Materials Science, Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI 48109, USA. E-mail: kotov@umich.edu

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include larger molecules, such as cyclodextrins or peptides, in which case they resemble GPs that bear sugars, lipids, and other groups added by posttranslational modification.

To what extent has similarity in surface properties led to analogs of biological functions? Some of these functions can indeed be found for NPs (see the figure, panel A). For instance, analogs of the bimolecular GroEL protein complex were found in cadmium selenide, gold, and nickel-palladium NPs (6). Bayraktar *et al.* observed formation of pair complexes of gold NPs with cytochrome c and cytochrome c peroxidase (3). Tuning the size and the charge should make it possible for the NP spheres to mimic either the cylindrical channel of GroEL or the semispherical binding patch of cytochrome c peroxidase.

Enzymatic activity has been observed in the NPs developed by Scrimin and co-workers, which have stabilizers including peptides forming a complex shell. For instance, successful hydrolysis of a phosphate bond of phosphodiester to create a functional replica of a ribonuclease can be achieved (7), and gold NPs modified with beta-cyclodextrin possessed esterase activity (8).

Recent studies also indicate that NPs can self-assemble into complex microscale superstructures such as chains, sheets, and twisted ribbons (9–11). Parallels can be made with GPs such as amelogenin (12) assembling in chains, S-layer proteins and chaperonin assembling in two-dimensional sheets (13), and GPs in the capsid of tobacco mosaic virus forming helical tubules (14). The accurate description of the self-assembly process was also achieved in computer simulations that incorporated force fields around the NPs similar to those used previously for proteins (9, 15). The similarity of the chemical behavior of NPs and GPs is not accidental but is based on analogous structure as well as thermodynamic and kinetic behavior of nanoscale structures in aqueous media.

Other emblematic functions of GPs have also been identified in recent publications but with lower degrees of experimental proof and functional resemblance (see the figure, panel B). For example, proteins can facilitate transport of DNA across cellular membranes. Bharali *et al.* found that NPs can efficiently do the same and cross cellular membranes themselves (16). They can support transfection of cells with genes replicating the function of bacterial SpoIIIE protein (17). The positive charge of the NPs and tight coiling of DNA around them resemble histone-DNA interactions and may account for the high efficiency of NPs as transfection vectors. Formation of extensive networks of NPs or gels

(18) can be compared with structural function of different proteins, but adequate replication of gel and network formation will require the demonstration of the reversibility of such reactions and different mechanical properties of the gels. Proteins perform a variety of functions when bound to DNA. Such binding occurs with NPs but without specificity (19).

It should be possible to replicate other functions of GPs that depend mainly on surface interactions by using NPs (see the figure, panel C). A logical extension of previous works is molecular engineering of the NP surface to reach specific and reversible binding of both small and large biomolecules, including DNA. These functions can find extensive use in biotechnology to control pathways of bacterial biosynthesis with NPs. Nanoscale systems with dynamic stimuli-responsive NP networks will lead to new sensing platforms and fluids with unusual flow responses reminiscent of many biological fluids. NP interactions with membrane receptors might have produced cell signaling events, but these effects remain to be investigated systematically (20, 21). Another potentially prolific direction is NP design to perform chiral catal-

ysis and inhibition of specific enzymes. If these functionalities are realized, they should enable medically relevant drug design, as well as clarifying the health effects of NPs present in the environment.

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PLANT SCIENCE

Communal Benefits of Transgenic Corn

Bruce E. Tabashnik

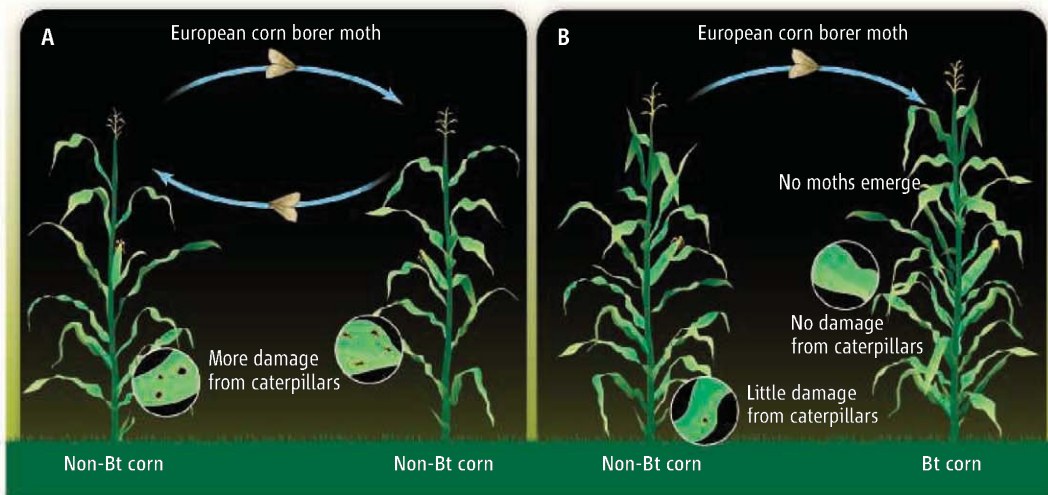
Genetically engineered corn plants can reduce pest damage on neighboring, unmodified plants.

Genetically engineered crops represent one of the most controversial and rapidly adopted technologies in the history of agriculture. First grown commercially in 1996, transgenic crops covered 135 million hectares (ha) in 25 countries during 2009 (1). To reduce reliance on insecticide sprays, corn and cotton have been genetically engineered to make insecticidal proteins derived from the common bacterium *Bacillus thuringiensis* (Bt). These Bt toxins kill some devastating insect pests, but unlike broad-spectrum insecticides, they do little or no harm to most other organisms, including people (2). Many pests have rapidly evolved resistance to insecticides, however, spurring concerns that adaptation by pests could quickly reduce the

efficacy of Bt crops and the associated environmental, health, and economic benefits (3–7). On page 222 of this issue, Hutchison *et al.* (8) rein in some of those concerns, documenting a landmark case in which Bt corn has remained effective against a major pest for more than a decade, yielding billions of dollars of estimated benefits to farmers in the midwestern United States.

Hutchison *et al.* describe Bt corn's suppression of the European corn borer (*Ostrinia nubilalis*), an invasive insect introduced into the United States in 1917. The caterpillars of this moth chew on leaves and tunnel in corn stalks. Before the advent of Bt corn, it caused losses of \$1 billion per year in the United States (8). However, susceptible caterpillars of this pest do not survive on Bt corn that produces toxins active against the larvae of Lepidoptera (moths and butterflies) (8).

Department of Entomology, University of Arizona, Tucson, AZ 85721, USA. E-mail: brucet@cal.s.arizona.edu



Halo effect. Bt corn planted near non-Bt corn can provide the unmodified plants with indirect protection from pests. European corn borer moths lay eggs indiscriminately on Bt corn and non-Bt corn, but their caterpillars survive and become moths only on non-Bt corn. (A) With only non-Bt corn, moths move between plants and lay eggs, and caterpillars damage plants. (B) Moths do not emerge from Bt corn plants, reducing the number of eggs and subsequent damage on non-Bt corn near Bt corn. Yield is highest for Bt corn (B), lowest for non-Bt corn (A), and intermediate for non-Bt corn near Bt corn (B).

Although it is not surprising that planting millions of hectares of Bt corn reduced the damage caused by the European corn borer, Hutchison *et al.* discovered that most of the economic benefits from 1996 to 2009 were associated with planting corn that does not make Bt toxins (i.e., non-Bt corn). This somewhat counterintuitive result arises from three facts: Farmers paid a premium of about \$10 to \$20 per ha for Bt corn seed; on average, non-Bt corn was more abundant than Bt corn; and pest populations decreased dramatically on non-Bt corn plants.

The suppression of pests on non-Bt plants near Bt plants—called the “halo effect”—was predicted on theoretical grounds by Alstad and Andow in 1996 (3). The halo effect occurs with European corn borer because females lay eggs indiscriminately on Bt and non-Bt corn, and the caterpillars hatching on Bt corn die (8) (see the figure). If Bt plants account for a substantial percentage of the available host plants, regional pest populations can be greatly reduced, resulting in less damage to non-Bt plants. Although the halo effect was seen before (6, 9, 10), Hutchison *et al.* are the first to report an economic analysis of this phenomenon based on large-scale, long-term data. They demonstrate that planting non-Bt corn pays off because farmers avoid the extra cost of Bt seed, yet still get some pest control benefits generated from neighboring Bt corn.

The immediate economic gains farmers reap from planting non-Bt corn could boost their compliance with the “refuge” strategy, which is designed to delay the evolution of pest resistance to Bt corn (3–5). In the United States, the Environmental Protection Agency

(EPA) requires farmers to plant refuges of non-Bt corn near Bt corn (2, 11). Refuges promote the survival of susceptible insects to mate with resistant insects that survive on Bt corn. If inheritance of resistance is recessive, the hybrid progeny from such matings will die on Bt crops, substantially slowing the evolution of resistance. This approach works best if the dose of toxin ingested by insects on Bt plants is high enough to kill all or nearly all of the hybrid progeny (12). Moreover, as the toxin dose increases, so too does the magnitude of resistance required for survival on Bt plants (13). Whereas mutations providing small decreases in susceptibility to Bt proteins are relatively common, those conferring sufficient resistance to enable survival on some types of Bt corn are exceedingly rare in the European corn borer (14, 15).

Although refuges have probably helped Bt crops remain effective longer than expected in most cases, some populations of at least four major pests have evolved resistance to Bt crops (12, 13, 16). Analyses of global resistance monitoring data suggest that the evolutionary principles underlying the refuge strategy can explain why some pest populations have evolved resistance faster than others (7, 12). In each case of field-evolved resistance to Bt crops, the high-dose standard was not met, refuges were scarce, or both (7, 12).

New tools to combat pest resistance to Bt crops include a wider array of toxins, including toxins genetically modified to counteract resistance (17). Also, to thwart resistance, plants that produce two or more distinct Bt toxins targeting the same pest are becoming increasingly important. For example, a

type of Bt corn registered in the United States in 2009 produces five distinct Bt toxins; three of these target caterpillar pests including European corn borer and two kill corn rootworm beetles (*Diabrotica* species) (11). Whereas the EPA had previously required non-Bt corn refuges planted in separate fields, rows, or strips, in April 2010 it approved sales of mixtures of corn seeds with and without Bt toxins that kill corn rootworms (11). This seed mixture approach ensures that farmers comply with the refuge strategy, and may be especially useful on small farms in developing countries where planting separate refuges is not practical. With the shift to seed mixtures and multitoxin

Bt corn, the EPA has dropped the minimum percentage of corn that farmers must plant in non-Bt corn refuges from 20% to as little as 10% (seed mixtures) or 5% (multitoxin plants) (11). No one knows how fast insects will adapt to Bt corn under these new conditions. As we scramble to stay one step ahead of the pests, let's keep in mind the groundbreaking report by Hutchison *et al.* affirming the adage that diversity breeds success.

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The Evolution and Future of Earth's Nitrogen Cycle

Donald E. Canfield,^{1*} Alexander N. Glazer,² Paul G. Falkowski³

Atmospheric reactions and slow geological processes controlled Earth's earliest nitrogen cycle, and by ~2.7 billion years ago, a linked suite of microbial processes evolved to form the modern nitrogen cycle with robust natural feedbacks and controls. Over the past century, however, the development of new agricultural practices to satisfy a growing global demand for food has drastically disrupted the nitrogen cycle. This has led to extensive eutrophication of fresh waters and coastal zones as well as increased inventories of the potent greenhouse gas nitrous oxide (N_2O). Microbial processes will ultimately restore balance to the nitrogen cycle, but the damage done by humans to the nitrogen economy of the planet will persist for decades, possibly centuries, if active intervention and careful management strategies are not initiated.

Nitrogen, the fifth most abundant element in our solar system, is essential for the synthesis of nucleic acids and proteins—the two most important polymers of life. Indeed, the nitrogen requirements for life are enormous; depending on the life form, for every 100 atoms of carbon incorporated into cells, between 2 and 20 atoms of nitrogen follow (1). The biogeochemistry of nitrogen is almost entirely dependent on reduction-oxidation (redox) reactions primarily mediated by microorganisms (2), and to a lesser extent on long-term recycling through the geosphere [e.g., (3)]. Despite the importance of nitrogen and its overwhelming abundance in the atmosphere, N_2 is virtually inert; hence, fixed inorganic nitrogen [most commonly nitrate (NO_3^-) and ammonium (NH_4^+) ions] often limits primary productivity in both marine and terrestrial ecosystems (2, 4, 5). Here, we review the nitrogen cycle on Earth, its evolutionary history, its interactions and feedbacks with other key elements, and the disruption of the cycle by humans over the past century.

What Is the Metabolic Basis of the Modern Nitrogen Cycle?

An active biosphere ultimately requires incorporation of nitrogen into biological molecules through nitrogen fixation, a process where prokaryotes in the bacterial and archaeal domains reduce nitrogen gas (N_2) to ammonium (Fig. 1). Some eukaryotes (e.g., legumes and termites) also support nitrogen fixation, but only in symbiotic association with nitrogen-fixing prokaryotes. Although the reduction of N_2 is an exergonic reaction, the activation energy required to break its $\text{N}\equiv\text{N}$ bond is formidable, and the reaction

requires a catalyst to overcome the energy barrier. The heterodimeric enzyme complex, nitrogenase, serves this role by hydrolyzing ~16 adenosine

triphosphate (ATP) molecules per molecule of N_2 fixed. Nitrogenase is an $\alpha_2\beta_2$ tetramer, in which each of the two α subunits catalyzes the ATP-dependent reduction of N_2 to NH_3 . In its most common form, each α subunit contains a MoFe₇S₉ metal cluster (the MoFe cofactor) that donates electrons to N_2 (6), with the electrons coming from the respiration of organic carbon. The genes encoding the two nitrogenase subunits are highly conserved but are widely dispersed across many phyla of bacteria and archaea, which suggests that nitrogen fixation evolved once and subsequently spread by vertical inheritance and by horizontal gene transfer (5, 7–9, 41).

Although nitrogenase is widely distributed among prokaryotic lineages, most organisms cannot fix nitrogen but rather obtain their nitrogen directly as NH_4^+ (or organic nitrogen) from the environment, or from the reduction of NO_3^- to NH_4^+ through assimilatory nitrate reduction. Both prokaryotes and eukaryotes are able to mediate

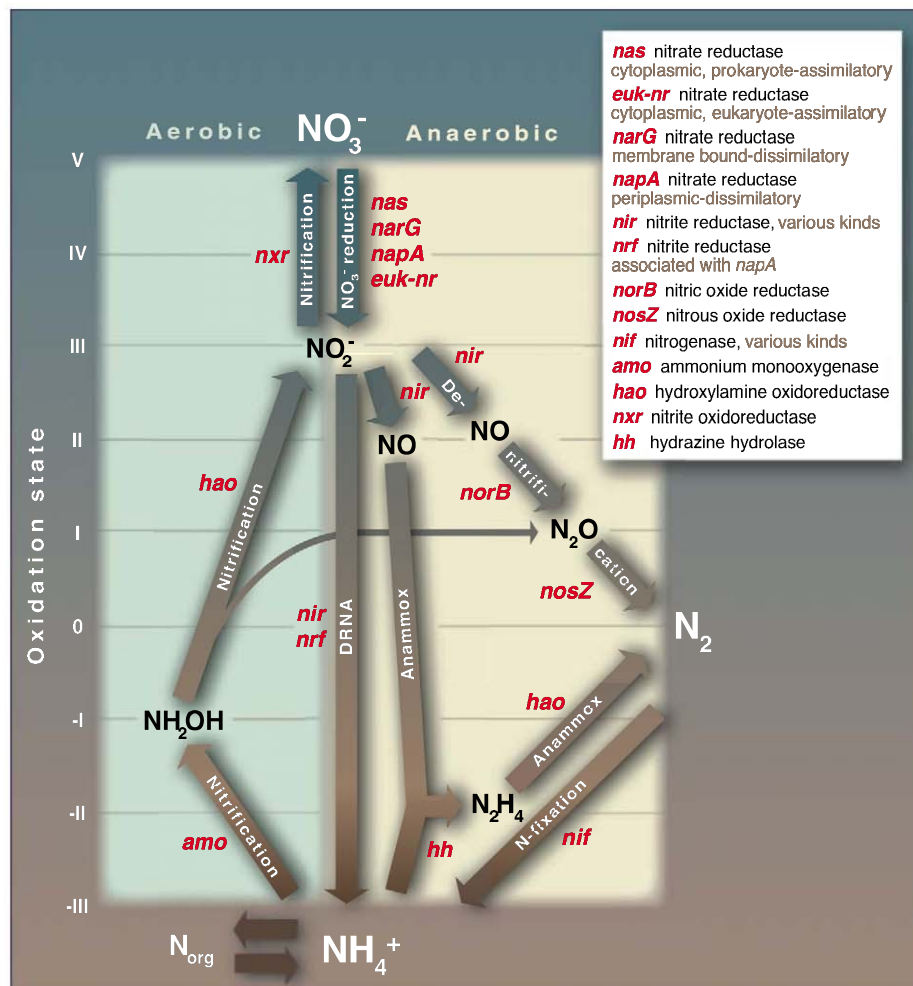


Fig. 1. The major biological nitrogen transformation pathways are linked by their associated enzymes [adapted from (63)]. Genes encoding enzymes that conduct the important transformations include those for various nitrate reductases (*nas*, *euk-nr*, *narG*, *napA*), nitrite reductases (*nir*, *nrf*), nitric oxide reductase (*norB*), nitrous oxide reductase (*nosZ*), nitrogenase (*nif*), ammonium monooxygenase (*amo*), hydroxylamine oxidoreductase (*hao*), nitrite oxidoreductase (*nxr*), and hydrazine hydrolase (*hh*).

¹Institute of Biology and Nordic Center for Earth Evolution, University of Southern Denmark, Campusvej 55, Odense M, Denmark. ²Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA. ³Institute of Marine and Coastal Studies and Department of Earth and Planetary Sciences, Rutgers University, New Brunswick, NJ 08901, USA.

*To whom correspondence should be addressed. E-mail: dec@biology.sdu.dk

this process. Ammonium is returned to the environment when organisms die, and its fate (and the variety of subsequent forms of nitrogen) depends on whether the local environment contains oxygen (Fig. 1). In the presence of oxygen, NH_4^+ is sequentially oxidized to NO_3^- by specific groups of bacteria and archaea. In this pathway, known as nitrification, organisms containing the enzyme ammonium monooxygenase first oxidize NH_4^+ to hydroxylamine, which is subsequently oxidized to NO_2^- by hydroxylamine oxidoreductase, and finally the NO_2^- is oxidized to NO_3^- by nitrite oxidoreductase (Fig. 1). The electrons and protons derived during ammonium and nitrite oxidation are used by the microbes to fix inorganic carbon in the absence of light (i.e., chemoautotrophy) (10). The greenhouse gas N_2O is a by-product in this process; indeed, nitrification from both marine and terrestrial environments is an important source of atmospheric N_2O (11, 12).

In the absence of oxygen, NO_3^- can be used by many microbes as a respiratory electron acceptor. Nitrate reduction is coupled to the anaerobic oxidation of organic carbon (Fig. 1), producing either NH_4^+ in a process known as dissimilatory nitrate reduction to ammonium (DNRA) or, more commonly, N_2 gas during denitrification (Fig. 1). Denitrifiers include representatives of more than 60 genera of Bacteria and Archaea, as well as some eukaryotes (e.g., fungi, protozoa, and benthic Foraminifera and Gromiida) (13, 14). The process involves four metalloenzymes: dissimilatory nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductases. N_2O is an obligate intermediate (Fig. 1), and some ultimately escapes to the atmosphere, making denitrification another important source of this greenhouse gas from both marine and terrestrial environments (15–17).

An alternative route from fixed nitrogen to N_2 is found among a group of bacteria known as a planctomycetes, where NH_4^+ oxidation is coupled to NO_2^- reduction in a process called anammox (anaerobic ammonium oxidation), an exergonic reaction used for chemoautotrophic growth (18). This process dominates N_2 production in many marine environments, but, unlike classical denitrification, it does not lead to the production of N_2O (18). Together, denitrification and anammox close the nitrogen cycle by returning N_2 gas back to the atmosphere (Fig. 1).

What Controlled Earth's Earliest Nitrogen Cycle?

The form(s) of nitrogen delivered during planetary accretion, the rate of accretion, and the secondary atmosphere arising from volcanism controlled the prebiotic nitrogen cycle. Planetary accretion models generally assume that nitrogen was delivered to the protoplanet as solid (ice) NH_3 , amino acids, and other simple organics. These reduced forms of nitrogen subsequently became oxidized via high-temperature reactions in the upper mantle with iron and other transition elements to form atmospheric N_2 , which outgassed from

volcanoes (19). Indeed, a sizable (but poorly constrained) proportion of the nitrogen on Earth is still associated with the mantle (Fig. 2). These abiotic processes are extremely slow, yielding an estimated turnover time for the nitrogen cycle of about 1 billion years (3).

Current models suggest that in the early Earth atmosphere, heat shock associated both with lightning (20, 21) and high-energy meteorite impacts (22) produced NO , which would have converted to NO_3^- and NO_2^- through a series of subsequent photochemical and aqueous phase reactions (23, 24). The conversion of N_2 to NH_3 by these processes was probably extremely slow, with estimates ranging from $\sim 2 \times 10^8 \text{ mol N year}^{-1}$ (assuming a low CO_2 atmosphere) to $\sim 2 \times 10^{10} \text{ mol N year}^{-1}$ (assuming a high- CO_2 atmosphere)

probably the most important (31). However, as a result of the limited H_2 supply and its escape to space, primary productivity was lower than contemporary rates by a factor of 1000 or more (Fig. 3) (31). Even so, heat shock processes may have kept pace with the demands of the emerging biosphere, especially if microorganisms evolved an early means (i.e., assimilatory nitrate reduction) to reduce any environmental NO_3^- to NH_4^+ within the cell.

Early in biological evolution, anoxygenic photosynthetic organisms evolved and harnessed the Sun's energy to oxidize substrates such as H_2 , H_2S , and Fe^{2+} (but apparently not NH_4^+) and to use the reducing equivalents for carbon fixation. If fixed inorganic nitrogen was not limiting in the earliest prephotosynthetic biosphere, it almost cer-

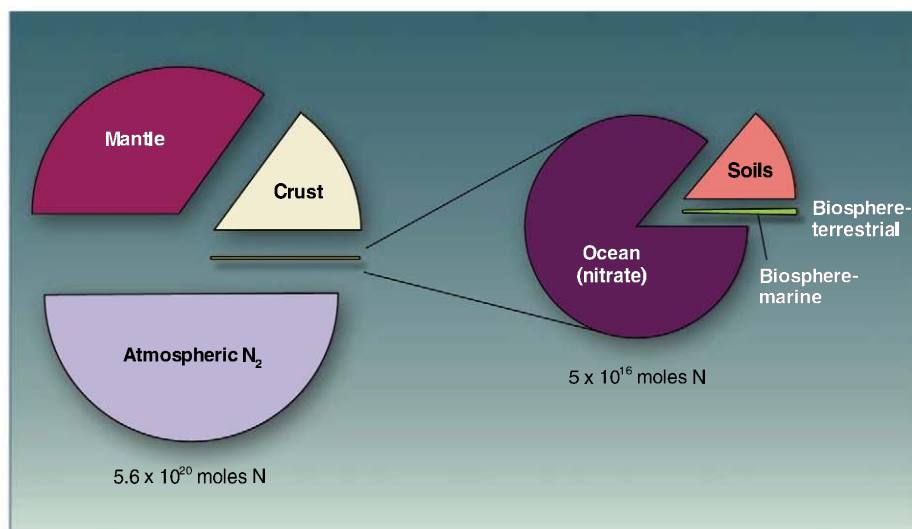


Fig. 2. The size of nitrogen reservoirs on Earth is highly variable. (44, 64–67).

(24). These estimates are lower than modern rates of biological nitrogen fixation in the oceans by a factor of 50 to 5000 (25). In principle, NO_3^- , NO_2^- , and N_2 can be abiotically reduced to NH_4^+ at high temperatures through interactions with metallic Fe, magnetite, or iron sulfide minerals (26, 27); however, no appreciable flux of NH_4^+ is apparent in modern hydrothermal vent systems where such reactions should occur. Nitrite is also readily reduced to NH_4^+ by Fe^{2+} at $\text{pH} > 7.3$ (28), which may have been of particular importance on the early Earth when the oceans were likely rich in Fe^{2+} (29). Because ultraviolet oxidation of atmospheric NH_3 (in equilibrium with NH_4^+ in the oceans) would have formed N_2 (30), N_2 gas remained the dominant form of nitrogen in the atmosphere (Fig. 3). Ammonium and possibly some NO_3^- dominated ocean chemistry, whereas NO_2^- would have been a minor phase (Fig. 3).

How Did the First Organisms Influence the Nitrogen Cycle?

The earliest organisms on Earth likely gained energy from chemically reduced compounds delivered from Earth's interior, of which H_2 was

tainly became limiting after the evolution of anoxygenic photosynthesis. Estimates suggest that anoxygenic phototrophs increased the flux of nutrients and carbon through the biosphere by a factor of up to 100 (31) (Fig. 3), with the oxidation of Fe^{2+} yielding potentially the highest rates of primary production. Nitrogen limitation would have provided strong selection pressure for the evolution of biological N_2 fixation to allow anoxygenic phototrophs to fully make use of the reducing substrates available to them in the environment. Indeed, the ability to fix N_2 evolved early in biological evolution (8), possibly in anaerobic photoautotrophs, all of which are in the domain Bacteria.

With anoxygenic photosynthesis, chemical stratification would have developed for the first time in the global ocean. Ammonium would have accumulated deep in the oceans, and intense productivity at the base of photic zone (analogous to the deep chlorophyll maximum in the modern oceans) would have removed both NH_4^+ and Fe^{2+} . Because primary production would have been limited by Fe^{2+} availability, a $\text{NH}_4^+/\text{Fe}^{2+}$ ratio of 2/3 in the deep waters would have been expected

from the stoichiometry of anoxygenic photosynthesis with Fe^{2+} (4 Fe^{2+} per CH_2O fixed as organic carbon), and a C/N ratio of 6/1 in organic matter. On the basis of this stoichiometry, and given estimated Fe concentrations of 40 to 120 μM in the deep ocean (29), NH_4^+ levels would have been 27 to 80 μM .

How Did the Nitrogen Cycle Respond to Changes in Earth's Surface Chemistry?

Both the evolutionary history of nitrogen metabolisms as well as their intensity depended on the evolution of Earth's surface chemistry. Hence, although the anaerobic process of nitrogen fixation likely evolved early in biological evolution, the efficiency of this process and its importance in regulating the nitrogen inventory of the oceans depended on the evolution of ocean chemistry (9, 32). Thus, before ~2.5 billion years ago, the oceans were rich in dissolved Fe^{2+} (29), which accumulated under very low atmospheric oxygen concentrations (33). Some nitrogen fixers contain paralogous genes that encode two alternative nitrogenases, where V or Fe replaces Mo. These alternative and less efficient forms are expressed when Mo is unavailable (34), and given the abundant availability of Fe on the early Earth and the lack of soluble Mo under conditions of low atmospheric oxygen, it is likely that the Fe form

dominated under these conditions. Indeed, the more efficient Mo form may not have become widely distributed until some 500 to 600 million years ago, after oxygenation of the deep ocean led to an increase in soluble Mo concentrations (35). All known nitrogenases, however, are irreversibly inhibited by molecular oxygen. Hence, to operate in the presence of oxygen, organisms evolved means to shield the enzyme complex by scavenging oxygen to low concentrations and/or by restricting its diffusion to the complex.

It is unclear when the other critical anaerobic processes in the nitrogen cycle, denitrification and anammox, evolved. If these processes evolved before oxygen-producing photosynthesis by cyanobacteria, they only became important after cyanobacterial evolution, because molecular oxygen appears to be requisite for the biological production of NO_3^- . Indeed, nitrification is the critical aerobic process in the nitrogen cycle (Fig. 1); once it evolved, the modern nitrogen cycle emerged.

The timing of cyanobacterial evolution is unclear, but periodic oxygenation of the surface environment occurred at least 200 to 300 million years before pronounced atmospheric oxygenation around 2.3 to 2.4 billion years ago [e.g., (33, 36, 37)]. Even after this, it was not until ~600 million years ago [e.g., (35, 38, 39)] that widespread oxygenation of the deep ocean occurred. During this

transition period of ~1.8 billion years, oxygenic phototrophs in the surface ocean resided above anoxygenic phototrophs at the transition between H_2S or Fe^{2+} in the deeper ocean layers (38–40). In such a situation, NH_4^+ would have been present in the deeper anoxic parts of the ocean, whereas NO_3^- and NO_2^- would have been concentrated at the oxic-anoxic transition as a result of nitrification just above and denitrification just below (Fig. 3). This situation is analogous to modern stratified basins such as the modern Black Sea, but some ventilation of the ocean interior may have accompanied the downwelling oxygen-enriched waters at high latitudes, as happens in the contemporary ocean.

While global rates of primary production increased markedly with the evolution of oxygenic photosynthesis (31) (Fig. 3), feedbacks among the nitrogen, carbon, and oxygen cycles potentially contributed to the retardation of Earth's surface oxidation. Thus, massive loss of fixed nitrogen from the oceans through anammox and denitrification are predicted during periods of deep-water anoxia (9, 31), potentially limiting the availability of fixed inorganic nitrogen. With nitrogen limitation, the burial of organic carbon would have been reduced, and hence net oxygen accumulation would have been retarded [i.e., a negative feedback (9, 31)].

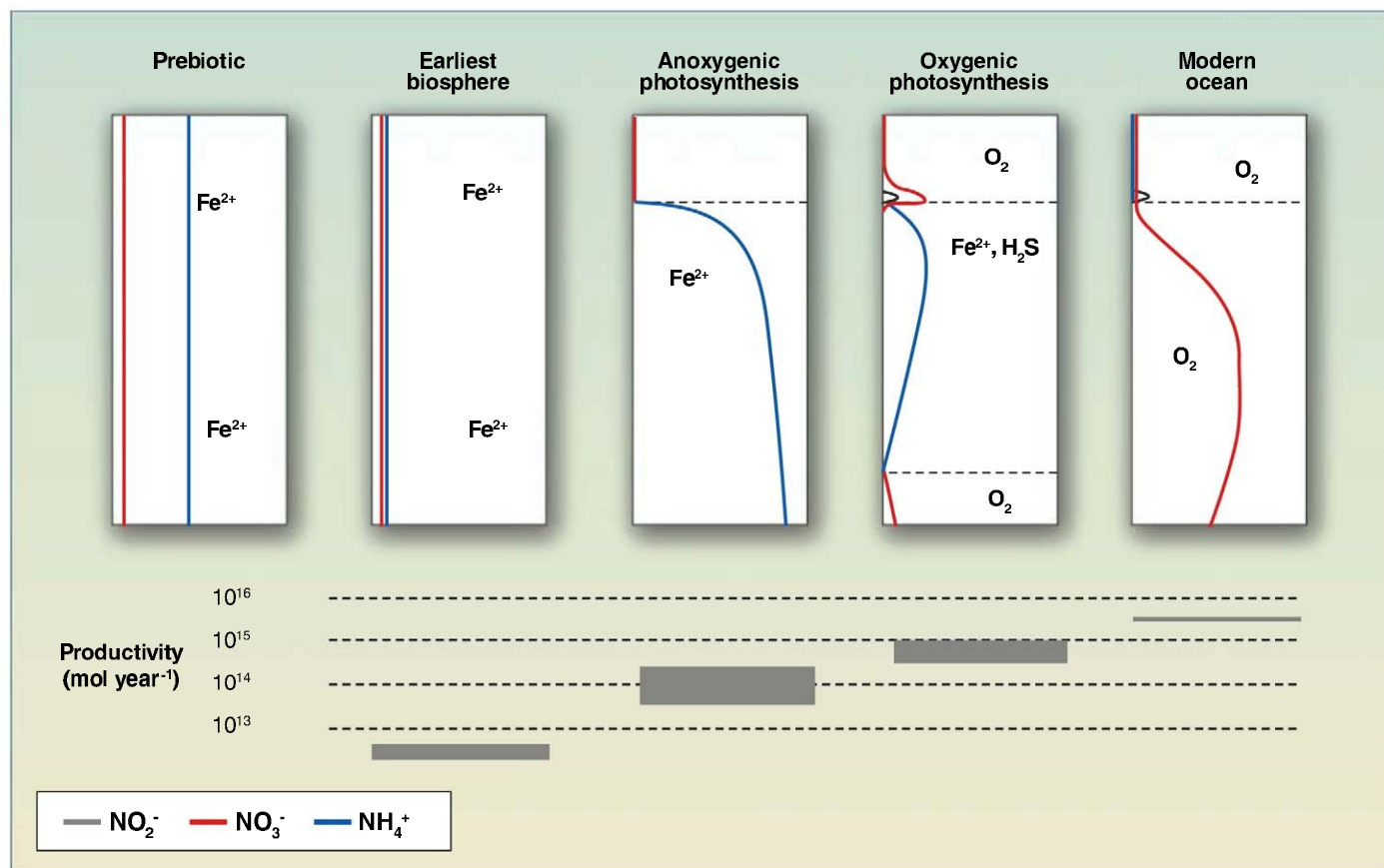


Fig. 3. The marine nitrogen cycle responded to changes to biological evolution and ocean chemistry through geologic time. The five major biological innovations are indicated by different vertical profiles of ocean chemistry. Rates of oceanic primary production (mol C year^{-1}) also increased as they changed in response to the evolving biosphere and the oxygenation of the oceans.

Stable isotopes provide some temporal constraints on the emergence of interactions between biogeochemical cycles. In the water column, both denitrification and anammox lead to an isotopic fractionation between the two stable isotopes of N; the lighter isotope, ^{14}N , is preferentially converted to N_2 , leaving the pools of fixed inorganic nitrogen enriched in ^{15}N . Indeed, isotopic analyses from late Archean shales (about 2.7 billion years ago) reveal organic matter with strong enrichments in ^{15}N (41), suggesting that the nitrogen cycle had either an anammox or classical denitrification pathway. The expression of these pathways required active nitrate production through nitrification, which suggests that molecular O_2 and the complete nitrogen cycle were present in the upper ocean for several hundred million years before the widespread oxygenation of the atmosphere (7).

How Did the Modern Nitrogen Cycle Evolve?

Oxygen rose to its modern levels over the last 550 million years [e.g., (42)], aided by the rise of terrestrial plants (43). With the oxygenation of the ocean interior, NO_3^- became the dominant nitrogen species, with minor concentrations of NH_4^+ and NO_2^- in the water column. On the modern Earth, rates of net primary production are nearly equally balanced between the land and sea at about $4 \times 10^{15} \text{ mol year}^{-1}$ each (44), and although current estimates are not well constrained, the natural, pre-industrial nitrogen cycles are also of a similar magnitude on land and in the sea (45) (Fig. 4).

A curious feature of the modern terrestrial nitrogen cycle is that denitrification and nitrogen fixation are largely balanced. A sizable fraction of the nitrogen transfer from the land to the sea results from a combined loss of anthropogenic nitrogen inputs through rivers and atmospheric transport of gaseous phases (e.g., NO_x , NH_3 , and N_2O) from the continents to the oceans (45, 46). There is apparently also a large annual storage of nitrogen on the continents (46). In the oceans, rates of denitrification appear to be higher than those of nitrogen fixation and terrestrial input, suggesting an imbalance in the system (45, 46) (Fig. 4). Overall, denitrification in the oceans is governed by oxygen supply (47). In coastal sediments, and in regions of low oxygen in the water column (such as in the eastern tropical Pacific, southwest Africa, and the Arabian Sea), denitrification is extensive. In contrast, N_2 fixation is primarily found in the tropical and subtropical regions of the Northern Hemisphere where continents deliver a biologically available dust source of iron (48). Assuming an average molar ratio for nitrogen and phosphate of 16/1 in organic matter

(i.e., the Redfield ratio), there is a NO_3^- deficit of $\sim 2 \mu\text{mol/liter}$ in the contemporary ocean, presumably reflecting a slight imbalance between N_2 fixation and denitrification (2). Because nitrification has a high absolute Fe requirement, and because Fe availability is variable from place to place in the oceans, nitrogen deficits may have been the norm in the global ocean, with nitrogen fixation lagging to fill the demands of the biosphere as necessitated by fixed nitrogen loss through denitrification.

Has Human Activity Created an Imbalance?

In the 20th century, humans began to have an enormous impact on the global nitrogen cycle by developing industrial processes to reduce N_2 to NH_4^+ , by implementing new agricultural practices that boost crop yields, and by burning fossil fuels (49). Agriculture alone contributes about

use efficiency is typically below 40%, meaning that most applied fertilizer either washes out of the root zone or is lost to the atmosphere by denitrification before it is assimilated into biomass. Given the rising costs of synthetic fertilizer production, this overuse is not only economically expensive, but also initiates a cascade of large-scale environmental impacts (52). Worldwide, nearly 90% of nitrogen fertilizer is NH_4^+ , where nitrifying bacteria can convert it to highly mobile NO_3^- , which in turn can leach into rivers, lakes, and aquifers. This results in nitrogen loss and leads to eutrophication of coastal waters, creating huge hypoxic zones around the world (53).

Under anoxic conditions (e.g., as found in wet soils), denitrification forms mainly N_2 but also forms N_2O , a fraction of which is lost to the atmosphere and increasingly contributes to the rise in atmospheric N_2O concentrations (54). As

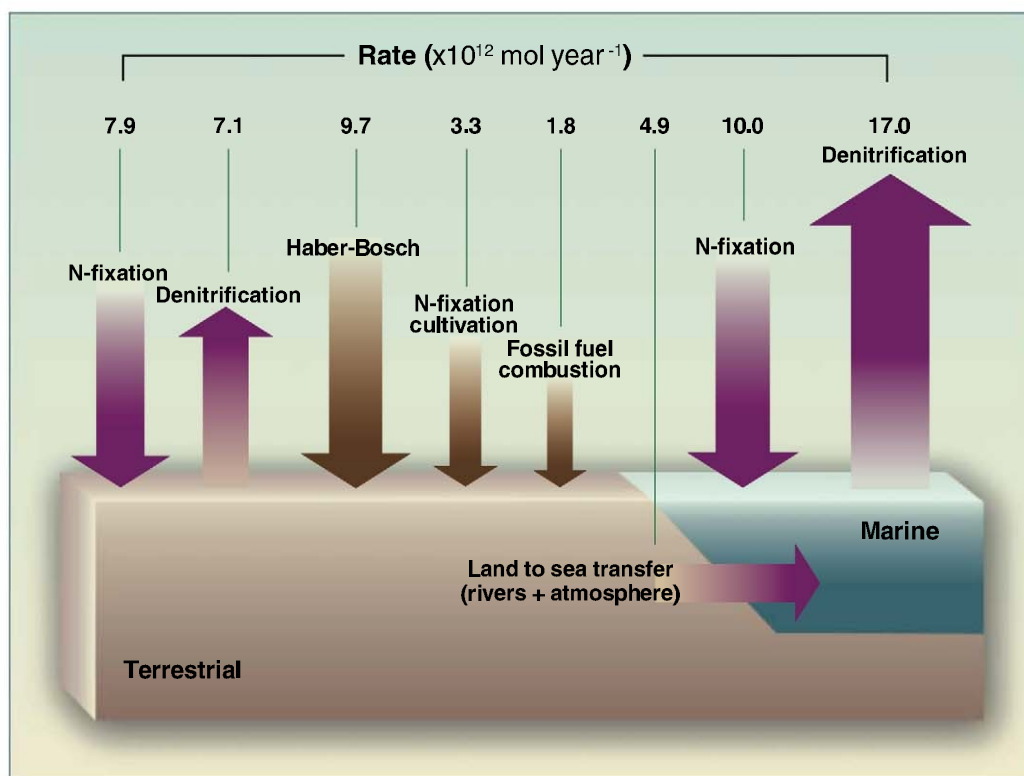


Fig. 4. Rates of nitrogen flux in the modern nitrogen cycle depend on the efficiency of the transformations between reservoirs. Arrow size reflects relative size of the flux. The dark brown arrows represent anthropogenic inputs (25, 45, 46, 52, 53, 68, 69).

$2.4 \times 10^{12} \text{ mol N year}^{-1}$ because of cultivation-induced nitrogen fixation, primarily from fodder legumes (46). During 2008 alone, the Haber-Bosch process of NH_4^+ production supplied $9.5 \times 10^{12} \text{ mol}$ (50), and fossil fuel combustion generated another $1.8 \times 10^{12} \text{ mol}$ (45). Together, anthropogenic sources contribute double the natural rate of terrestrial nitrogen fixation, and they provide around 45% of the total fixed nitrogen produced annually on Earth (Fig. 4).

From 1960 to 2000, the use of nitrogen fertilizers increased by $\sim 800\%$ (51), with wheat, rice, and maize accounting for about 50% of current fertilizer use. For these crops, the nitrogen

a greenhouse gas, N_2O has 300 times (per molecule) the warming potential of CO_2 , and it also reacts with and destroys ozone in the stratosphere (55). Because nitrification also produces N_2O as an intermediate, agricultural systems represent huge sources of N_2O to the atmosphere, accounting for about one-quarter of global N_2O emissions (56).

What Will the Future Nitrogen Cycle Look Like?

Humans may have produced the largest impact on the nitrogen cycle since the major pathways of the modern cycle originated some 2.5 billion

years ago. Natural feedbacks driven by micro-organisms will likely produce a new steady state over time scales of many decades (i.e., excess nitrogen added from human sources will no longer accumulate, but will be removed at rates equivalent to rates of addition). However, because of the projected increase in human population through at least 2050, there will be demand for a concomitant increase in fixed nitrogen for crops to feed this population. One potential consequence of increased fixed nitrogen use will be increased fluxes of riverine nitrogen to coastal zones (57), leading in turn to enhanced biological productivity, increased coastal anoxia, detrimental impacts on water quality, and increased fluxes of N_2O to the atmosphere.

Several new approaches and a much wider use of more sustainable time-honored practices, however, can decrease nitrogen use substantially. These include (i) systematic crop rotation [e.g., legume cropping in maize-based systems supplies the nitrogen otherwise provided by synthetic fertilizers (58)], (ii) optimizing the timing and amounts of fertilizer applied to increase the efficiency of their use by crops (59), (iii) breeding or developing genetically engineered varieties for improved nitrogen use efficiency (60), (iv) improving the ability of economically important varieties of wheat, barley, and rye to produce nitrification inhibitors through traditional breeding techniques (60, 61), and (v) further developing cereals and other crops with endosymbiotic nitrogen-fixing bacteria to supply their nitrogen needs [e.g., (62)]. Market forces may drive these improvements because the rising economic and environmental costs of nitrogen fertilizers will accelerate a demand for increased nitrogen use efficiency in agriculture (17). Thus, humans can do something about managing the nitrogen cycle, and microbial processes will ensure that a new balance in the cycle will be reached. However, even with management, the future cycle will likely be different from the one that preceded the Industrial Revolution.

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Bacteria Use Type IV Pili to Walk Upright and Detach from Surfaces

Maxsim L. Gibiansky,^{1*} Jacinta C. Conrad,^{2*} Fan Jin,¹ Vernita D. Gordon,¹ Dominick A. Motto,⁴ Margie A. Mathewson,³ Wiktor G. Stopka,³ Daria C. Zelasko,³ Joshua D. Shrout,⁴ Gerard C. L. Wong^{1,3†}

Bacterial biofilms are multicellular surface-bound communities with important human health impacts (1). For *Pseudomonas aeruginosa*, a biofilm-forming pathogen responsible for lethal infections in cystic fibrosis (2), the motility appendages type IV pili (TFP) mediate the “twitching” motility mode observed in biofilms (3). Comparatively little is known about the transition from planktonic to surface-associated states that initiates biofilm formation. Here, we converted microscopy movies into searchable databases of bacterial behavior by using particle-tracking algorithms for quantitative analysis at fast time scales of all visible bacterial trajectories (4).

Shortly after attachment, before microcolony formation, we observed two TFP-driven surface motility mechanisms. Flagella-deficient Δ *flhM* mutants, whose movement is strictly TFP-dependent, “crawled” along their body axis with high directional persistence when oriented horizontally, parallel to the surface, and “walked” omnidirectionally with low directional persistence when attached vertically by one end (Fig. 1, A and B). Bacteria reversibly transitioned between these mechanisms. By contrast, *Mycobacterium xanthus*

slowly jiggles vertically before transitioning to a horizontal orientation for lateral crawling (5).

Each mechanism confers advantages for surface exploration (6). From the ensemble-averaged mean-square displacement, we found that vertical walking bacteria moved nearly diffusively, whereas horizontal bacteria could be divided into crawling superdiffusive and surface-anchored subdiffusive subpopulations (fig. S1). The average persistence length, L_p , over which trajectories appear straight was shorter for walking bacteria (2 μ m) than for crawling bacteria (6 μ m); the former was similar to extension distances of TFP (7), suggesting that walking was caused by pulls of splayed TFP. Walking bacteria exhibited a higher instantaneous velocity [mean 71 ± 2 nm/s (SEM) versus 41 ± 2 nm/s], but crawling bacteria moved further on long time scales because of the L_p . Crawling enabled directional motion; walking enabled rapid local exploration. These trends were preserved in wild-type (WT) bacteria.

Bacterial orientation played a key role in life cycle events. In 99% of 214 WT division events, one daughter cell remained attached horizontally; the majority (67%) of the other daughters left the

division site by detaching, walking, or crawling. TFP governed this motility; TFP-deficient bacteria did not move apart after division. By using our search engine to locate all detachment events, we observed that detaching bacteria were overwhelmingly oriented out of plane (Fig. 1C). We found that TFP facilitated detachment by tilting from horizontal to vertical orientations; the influence of prevailing conditions was weak. TFP-deficient Δ *pilA* bacteria were defective in making this transition. This suggests a physical onset of biofilm formation, mediated by the transition from reversible polar attachment to irreversible longitudinal attachment (8).

Bacteria lacking TFP neither crawled nor achieved vertical orientations for walking and detachment. Δ *pilA* biofilms contained heterogeneous bacterial clusters (6) whose positions were determined by initial attachment sites (Fig. 1D). Divisions ($N_d = 79$) outnumbered attachments ($N_a = 18$) during 1 hour of cluster formation, indicating that clusters primarily grew via division. TFP-competent WT could actively walk, crawl, redistribute, and detach; despite a similar number of divisions ($N_d = 95$), the WT biofilm did not contain clusters, indicating the Δ *pilA* biofilm morphology is caused by motility defects rather than adhesion defects.

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Fig. S1
References

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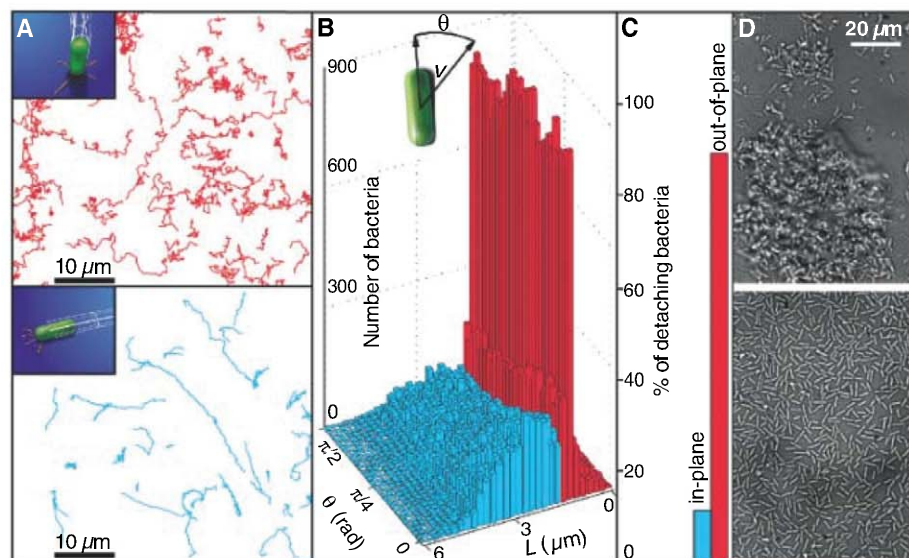


Fig. 1. TFP-mediated (A) vertical walking (top) and horizontal crawling (bottom) trajectories in *P. aeruginosa*. (B) Two-dimensional histogram of the angle between the bacterial axis and velocity (θ) and projected length (L) of $N = 70,073$ bacterial images showing populations of vertical (red) and horizontal (blue) bacteria. (C) Out-of-plane (vertical or tilted) orientations facilitate detachment. (D) Δ *pilA* biofilms had clusters (top); WT biofilms were uniform (bottom).

¹Department of Bioengineering, California Nano Systems Institute, University of California, Los Angeles, CA 90024, USA.

²Department of Chemical and Biomolecular Engineering, University of Houston, Houston, TX 77204, USA. ³Department of Materials Science and Engineering, University of Illinois, Urbana-Champaign, IL 61801, USA. ⁴Department of Civil Engineering and Geological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA.

*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: gclwong@seas.ucla.edu

Tau Reduction Prevents A β -Induced Defects in Axonal Transport

Keith A. Vossel,^{1,2*} Kai Zhang,³ Jens Brodbeck,¹ Aaron C. Daub,¹ Punita Sharma,¹ Steven Finkbeiner,^{1,2} Bianxiao Cui,³ Lennart Mucke^{1,2*}

Amyloid- β (A β) peptides, derived from the amyloid precursor protein (APP), and the microtubule-associated protein tau are key pathogenic factors in Alzheimer's disease (AD). However, the exact mechanisms by which they impair cognitive functions are unknown. Human APP (hAPP) transgenic mice have high A β concentrations in the brain and develop aberrant neuronal activity and behavioral deficits (1, 2). Lowering endogenous tau prevents these abnormalities without affecting baseline neuronal functions (1, 2). The mechanisms of this rescue are unknown. Axonal transport is critical for neuronal function and is impaired by A β (3–6). Whether tau also affects axonal transport is controversial (7–9).

To explore whether tau reduction prevents A β -induced defects in axonal transport, we studied axonal transport of mitochondria and the neurotrophin receptor TrkA, whose neuronal distributions are altered in AD (10, 11). Hippocampal neuronal cultures from tau-deficient (*Tau*^{−/−} or *Tau*^{+/-}) mice (1) and wild-type (*Tau*^{+/+}) controls were transfected with plasmids expressing fluorescent markers of mitochondria [mito-red fluorescent protein (RFP)] or TrkA (TrkA-mCherry). At 10 to 14 days in vitro, cargo motility (moving cargoes/total cargoes) and velocity (μ m/s) were assessed before and after treatment with A β ₁₋₄₂ oligomers (2 μ M) (12). A β rapidly inhibited axonal motility of mitochondria and TrkA in wild-type neurons. The effect was stronger on anterograde (mitochondria, $P < 0.001$; TrkA, $P < 0.001$) than retrograde (mitochondria, $P < 0.01$; TrkA, nonsignificant) transport. Complete or partial tau reduction prevented these defects without affecting axonal transport at baseline (Fig. 1 and movies S1 to S6). Moving cargoes had similar velocities in all *Tau* genotypes at baseline and after A β treatment.

Tau reduction did not enhance axonal transport under physiological conditions. However, tau levels were more critical for axonal transport in the presence of A β . A β oligomers impair axonal motility of cargoes through complex mechanisms involving *N*-methyl-D-aspartate receptor signaling (3), activation of glycogen synthase kinase 3 β (3, 4) and casein kinase 2 (5), and actin polymerization (6). Why A β requires tau to impair axonal transport is uncertain; tau might interact directly or indirectly with any of these pathways or enhance the effects independently by competing with motor proteins for microtubule access (7). Although most concentrated in axons, tau may have A β -enabling activities also in dendrites (2).

Tau ablation induces axonal spheroids in Tg2576 APP transgenic mice (13), but the functional im-

portance is unknown. Tau reduction prevents A β -induced neuronal and behavioral deficits in hAPPJ20 mice (1) and APP23 mice (2). Protection against A β -induced defects in axonal transport is one of several possible mechanisms for this rescue.

Although tau did not affect axonal transport under baseline untreated conditions in vitro (this study) or in vivo (9), it may still be important under physiologic conditions. For example, local tau gradients may promote cargo detachment at strategic points (7, 13). Tau may also regulate cel-

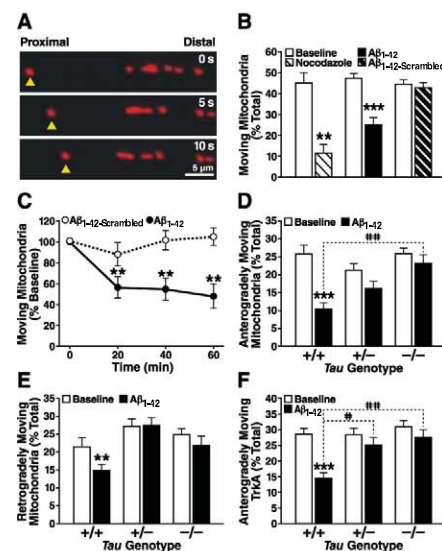


Fig. 1. (A) Anterograde axonal movement of a single mitochondrion (yellow triangles) is shown in successive 5-s image frames. (B) The microtubule-depolymerizing agent nocodazole (10 μ g/ml) and oligomeric A β ₁₋₄₂ (2 μ M) inhibited mitochondrial movements in *Tau*^{+/+} axons within 60 min. A β with a scrambled amino acid sequence (A β ₁₋₄₂-Scrambled, 2 μ M) had no effect. $n = 7$ to 24 axons per condition. ** $P < 0.01$, *** $P < 0.001$ versus corresponding baseline (paired *t* tests with Bonferroni correction). (C) A β ₁₋₄₂ inhibited mitochondrial movements in *Tau*^{+/+} axons within 20 min. $n = 7$ to 8 axons for each data point. ** $P < 0.01$ versus corresponding baseline (paired *t* tests, Bonferroni). (D to F) A β ₁₋₄₂ inhibited mitochondrial [(D) and (E)] and TrkA (F) motility in *Tau*^{+/+} axons within 60 min but not in *Tau*^{+/-} or *Tau*^{−/−} axons. $n = 24$ to 45 axons for each genotype and condition. ** $P < 0.01$, *** $P < 0.001$ versus corresponding baseline (paired *t* tests, Bonferroni). The percent reduction in anterograde transport in the presence of A β was greater in *Tau*^{+/+} axons than in *Tau*^{+/-} or *Tau*^{−/−} axons [(D) and (F)]. # $P < 0.05$, ## $P < 0.01$ (Kruskal-Wallis analysis of variance, Dunn). Error bars are SEM. See also table S1.

lular transport of its binding partners (2), and tau reduction might have affected axonal transport of cargoes we did not assess. Partial tau reduction may strike a balance between therapeutic safety and efficacy because it prevented A β -induced axonal transport defects as well as aberrant neuronal activity (1), cognitive deficits (1), and premature mortality (1, 2) in hAPP mice. In addition to tau reduction strategies, components of the axonal transport machinery and of A β - or tau-related signaling cascades are potential therapeutic targets warranting further investigation.

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¹Gladstone Institute of Neurological Disease, San Francisco, CA 94158, USA. ²Department of Neurology, University of California, San Francisco, CA 94158, USA. ³Department of Chemistry, Stanford University, Stanford, CA 94305, USA.

*To whom correspondence should be addressed. E-mail: kvossel@gladstone.ucsf.edu (K.A.V.); lmucke@gladstone.ucsf.edu (L.M.)

Nanophase Transition Metal Oxides Show Large Thermodynamically Driven Shifts in Oxidation-Reduction Equilibria

Alexandra Navrotsky,* Chengcheng Ma, Kristina Lilova, Nancy Birkner

Knowing the thermodynamic stability of transition metal oxide nanoparticles is important for understanding and controlling their role in a variety of industrial and environmental systems. Using calorimetric data on surface energies for cobalt, iron, manganese, and nickel oxide systems, we show that surface energy strongly influences their redox equilibria and phase stability. Spinel (M_3O_4) commonly have lower surface energies than metals (M), rocksalt oxides (MO), and trivalent oxides (M_2O_3) of the same metal; thus, the contraction of the stability field of the divalent oxide and expansion of the spinel field appear to be general phenomena. Using tabulated thermodynamic data for bulk phases to calculate redox phase equilibria at the nanoscale can lead to errors of several orders of magnitude in oxygen fugacity and of 100 to 200 kelvin in temperature.

Differences in surface energies alter relative free energies of polymorphs (materials with different crystal structures but the same composition), which cause size-driven thermodynamic crossovers in phase stability at the nanoscale (1–4). Because oxyhydroxides generally have smaller surface energies than oxides, dehydration reactions ($2FeOOH \rightarrow Fe_2O_3 + H_2O$) shift to higher temperatures by as much as 100 K at the nanoscale (4). These shifts in phase stability change what materials form under given conditions (pressure, temperature, or humidity) and affect physical properties and chemical reactivity. Here, we document similarly large thermodynamic shifts in the positions of oxidation-reduction (redox) equilibria in oxygen fugacity-temperature space for nanoscale transition metal oxides (5).

The Co-O system was chosen for detailed study because both Co(II) oxide (rocksalt CoO) and Co(II, III) oxide (Co_3O_4 spinel) can be prepared and characterized as both bulk and nanoscale materials, and the surface energy of CoO has already been measured (6). There is recent interest in the Co-O system for water splitting (7, 8), but it also serves as a useful model for other transition metal-oxygen systems, such as Fe-O, Mn-O, and Ni-O. Using calorimetric methods (5), we determined the surface energies for hydrated and anhydrous surfaces of CoO and Co_3O_4 . To complement the Co-O data, we also examined geochemically and technologically relevant metal oxides, including NiO, Fe_3O_4 , Mn_2O_3 , and Mn_3O_4 (5).

According to the surface energies in Table 1, calculated changes in enthalpy occur relative to

the bulk phase with increasing surface area (Fig. 1). The surface energy of oxides is based on calorimetric data, whereas that of anhydrous Co metal is based on computation (9), and that of the hydrated metal surface is approximated as 75% that of the anhydrous surface. This is roughly the ratio for oxides, but metal surfaces probably hy-

drate less strongly than oxides, so the actual difference may be less for metals. Thus, the difference in surface energy for hydrated surfaces between metal and oxide may be even greater than this estimate, which would make the true shifts in redox equilibria even greater. A steeper slope in Fig. 1 indicates a larger surface energy and a greater destabilization for small particles. CoO is thus more destabilized than either Co or Co_3O_4 for a given surface area. The Fe-O system behaves similarly (Fig. 1).

A number of spinel phases have lower surface energies than other oxides of the same metal (Table 1). Previous work showed that the lower surface energy of the defect spinel forms of Al_2O_3 and Fe_2O_3 relative to the corundum form causes a crossover in phase stability at the nanoscale (1, 10). Data on $MgAl_2O_4$ (11) and Co_3O_4 , Fe_3O_4 , and Mn_3O_4 also confirm low surface energies for these spinels (Table 1). Thus, it seems probable that other transition metal spinels (such as $CoFe_2O_4$, $NiFe_2O_4$, $CoMn_2O_4$, and perhaps even the lithium-bearing spinels used in batteries) have low surface energies as compared with those of more oxidized and more reduced phases, and the size-driven changes in redox equilibria may be general phenomena.

The observed differences in surface energies shift redox free energies by 10 to 30 kJ/mol, oxygen fugacity by several orders of magnitude,

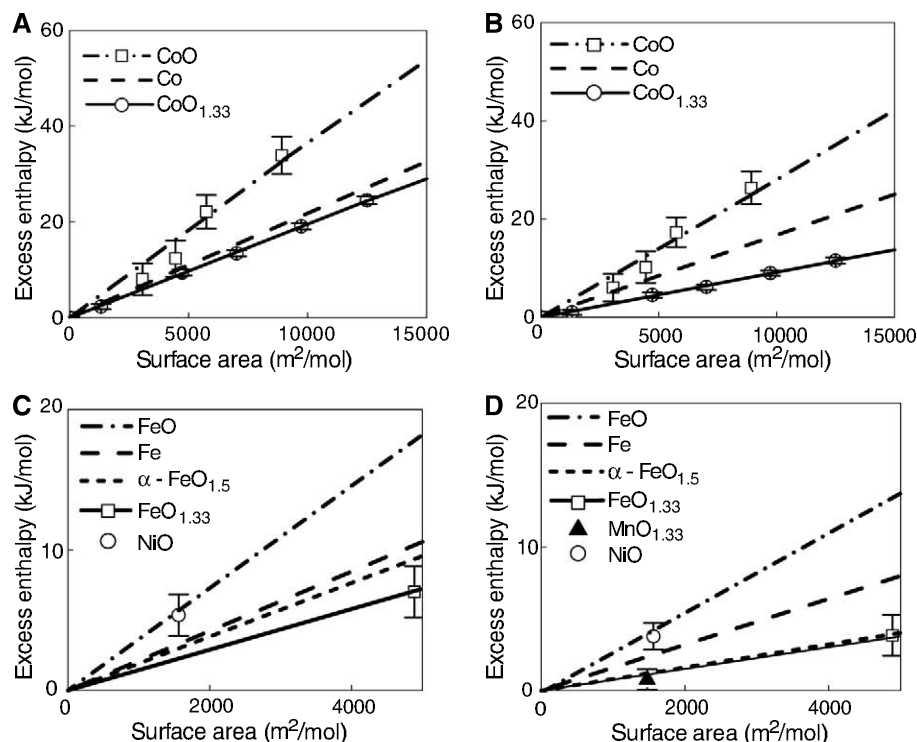


Fig. 1. Enthalpy relative to bulk phase and liquid water (excess enthalpy in kJ/mol, caused by increase in surface area), plotted versus surface area for (A) Co-O phases with anhydrous surfaces, (B) Co-O phases with hydrated surfaces, (C) Fe-O phases with anhydrous surfaces, and (D) Fe-O phases with hydrated surfaces. Oxide data (Co_3O_4 , Fe_3O_4 , Mn_3O_4 , and Fe_2O_3) in table S1 are converted to enthalpy per mole of $CoO_{1.33}$, $FeO_{1.33}$, $MnO_{1.33}$, and $FeO_{1.5}$ along with Co and Fe to maintain 1 mol metal stoichiometry for clear comparison of all phases.

Peter A. Rock Thermochemistry Laboratory and Nanomaterials in the Environment, Agriculture, and Technology Organized Research Unit, University of California at Davis, Davis, CA 95616, USA.

*To whom correspondence should be addressed. E-mail: anavrotsky@ucdavis.edu

or temperature by 100 to 200 K for 10-nm-diameter particles. A general formulation of the effect of particle size on chemical equilibria among solid phases is that systems favor phase assemblages of lower surface energy to counteract the high surface areas that are inherent to nanoparticles.

The calculated Co-O phase diagram for 10-nm nanoparticles confirms a much diminished stability field for the divalent oxide (Fig. 2). This narrowing is consistent with several qualitative observations made during the synthesis and handling of transition metal oxide nanoparticles. Decomposition of CoCO_3 in vacuum usually yields

Co_3O_4 unless the reaction occurs at high temperature, in which the divalent oxide coarsens (12). Size-driven oxidation by CO_2 also occurs when decomposing MnCO_3 and FeCO_3 (13). At a relatively high CO/CO_2 ratio with little oxygen supply, nano- Co_3O_4 —which we argue to be thermodynamically stabilized at the nanoscale under catalytic conditions—makes an excellent catalyst for low-temperature CO oxidation (14). Indeed, understanding nanoscale redox shifts in these systems may hold a key to understanding and designing efficient catalysts, including cobalt-based nanocluster catalysts for the splitting of water (7, 8). We observed that CoO nanoparticles

smaller than 8 nm, when dropped into water, evolved a gas that was shown to be hydrogen by its flammability. Such spontaneous oxidation of Co^{2+} and reduction of H_2O is not seen for larger particles. We also observed that 13-nm Mn_2O_3 particles, when heated in air at 975 K for 4 hours, are partially reduced with the appearance of small amounts of Mn_3O_4 (5), whereas 38-nm particles are not reduced. Such reduction is thermodynamically spontaneous in air for coarse material only above 1138 K according to standard thermodynamic data (15). These observations provide further evidence for shifts in the free energies of redox reactions at the nanoscale.

Using the values for surface energies in Table 1 and thermodynamic data for the bulk Fe-O system (5), the phase equilibria involving metal Fe, wustite $\text{Fe}_{0.947}\text{O}$, magnetite Fe_3O_4 , and hematite $\alpha\text{-Fe}_2\text{O}_3$ were computed (Fig. 2). The redox chemistry is altered drastically at the nanoscale, with the elimination of $\text{Fe}_{0.947}\text{O}$ as a stable phase, substituted by direct equilibrium between Fe and Fe_3O_4 . $\text{Fe}_{0.947}\text{O}$ has a low-temperature stability limit of 850 K relative to Fe and Fe_3O_4 in the bulk (15). At the nanoscale, this temperature greatly increases. The calculations imply that $\text{Fe}_{0.947}\text{O}$ nanoparticles are unlikely to form below 100 nm in size because the lowest temperature at which they would be stable with respect to $\text{Fe}_3\text{O}_4 + \text{Fe}$, ~ 1000 K, is high enough that coarsening would be rapid. Particles below ~ 16 nm in diameter would not be stable up to the melting point of bulk $\text{Fe}_{0.947}\text{O}$.

The interpretation of the conditions of formation of Fe-O phase assemblages in technological, geological, and planetary environments must be done in the context of the effect of surface energy on the position of the redox boundaries. Wustite nanoparticles of a few nanometers in diameter produced by high-energy ball milling (16) spontaneously oxidize, starting at the surface, to Fe_3O_4 . This creates a core-shell structure with exchange anisotropy that is critical for hard disk read-head and sensor applications (17, 18). We argue that the surface thermodynamics drives the formation of such technologically useful multiphase nanocomposite structures.

Redox and dissolution reactions of iron and manganese oxides provide energy sources for microbial communities (19). For example, Fe(II) -rich mineral dissolution is usually accompanied by oxidation, which supports Fe-oxidizing microorganisms (20). Shifts in thermodynamics at the nanoscale will change the free energy of such reactions. It is conceivable that organisms tailor particle size to optimize both the kinetics and the free energy change available to them. It is also possible that nanoparticle redox reactions, with energetics different from the bulk, may have played a role in chemical processes leading to abiotic organic synthesis and the origin of life on the early Earth.

The smaller range of stability of divalent transition metal oxides at the nanoscale also implies easier reduction to metal in vacuum. Small par-

Table 1. Surface energies for transition metal oxides and related systems.

Phase	Surface energy (anhydrous surface) (J/m^2)	Surface energy (hydrated surface) (J/m^2)
Co (hcp)	2.22 ± 0.30 (9)	$1.66 \pm 0.23^*$
Fe (bcc)	2.12 ± 0.29 (9)	$1.59 \pm 0.22^*$
$\text{Fe}_{0.947}\text{O}$ (rocksalt)	$[3.6]^\dagger$	$[2.8]^\dagger$
CoO (rocksalt)	3.57 ± 0.30	2.82 ± 0.20 (6)
$\text{NiO}^\ddagger$ (rocksalt)	3.5 ± 0.5	2.4 ± 0.4
Co_3O_4 (spinel)	1.96 ± 0.05	0.92 ± 0.04
$\text{Fe}_3\text{O}_4^\ddagger$ (spinel)	1.44 ± 0.30	0.79 ± 0.28
$\text{Mn}_3\text{O}_4^\ddagger$ (spinel)	—	0.5 ± 0.4
$\gamma\text{-Fe}_2\text{O}_3$ (spinel)	0.71 ± 0.13 (10)	0.57 ± 0.10 (10)
$\gamma\text{-Al}_2\text{O}_3$ (spinel)	1.53 ± 0.40 (25)	0.7 ± 0.4 (25)
MgAl_2O_4 (spinel)	$1.8 \pm 0.3^\S$	$0.9 \pm 0.3^\S$
Fe_2O_3 (hematite)	1.9 ± 0.3 (26)	0.75 ± 0.16 (27)
$\text{Mn}_2\text{O}_3^\ddagger$ (bixybite)	—	1.66 ± 0.21

*Energy of hydrated metal surface is assumed to be 0.75 that of the anhydrous surface, as discussed in the text. † The similarity of values for CoO and NiO suggests that it is reasonable to assign a similar surface energy to wustite, $\text{Fe}_{0.947}\text{O}$. Changes in the oxygen nonstoichiometry of wustite at the nanoscale are not considered. Neglecting such variations does not significantly change the positions of the redox equilibria. ‡ For calorimetric data, see table S1. § Calculated using data from previous study (11).

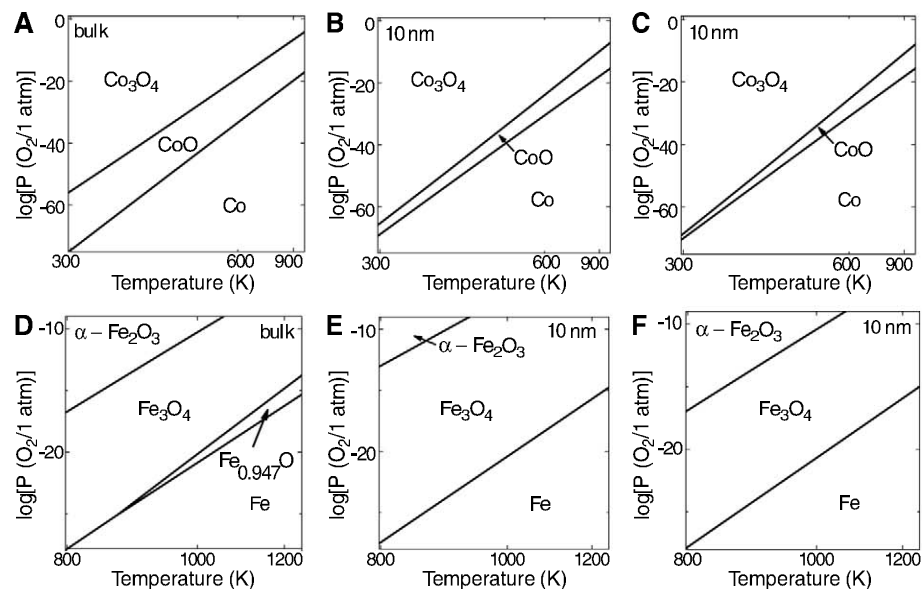


Fig. 2. Calculated phase diagrams for bulk and 10-nm-diameter spherical particles. (A) Co-O (bulk). (B) Co-O (anhydrous 10-nm particles). (C) Co-O (hydrated 10-nm particles). (D) Fe-O (bulk). (E) Fe-O (anhydrous 10-nm particles). (F) Fe-O (hydrated 10-nm particles). The temperature scale is in $1/T$, in order to make a linear plot, although the labeled temperatures are in T (kelvin). There is no stability field for 10-nm wustite $\text{Fe}_{0.947}\text{O}$.

ticles of hydrated NiO and NiFe₂O₄ form Ni metal when examined by means of transmission electron microscopy (21, 22). There are reports of unusual magnetism in semiconductors (ZnO and TiO₂) doped with cobalt (23, 24). The crystallographic position, clustering, phase separation, and oxidation state of the dopant are critical to understanding the origin of the reported ferromagnetism. Changes in the Co-O phase diagram at the nanoscale present additional variables, particularly because many measurements of spectroscopic, structural, and physical properties are done under vacuum conditions that may produce Co metal more readily in nanoscale systems.

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Tracking Hydrocarbon Plume Transport and Biodegradation at Deepwater Horizon

Richard Camilli,^{1*} Christopher M. Reddy,² Dana R. Yoerger,¹ Benjamin A. S. Van Mooy,² Michael V. Jakuba,³ James C. Kinsey,¹ Cameron P. McIntyre,² Sean P. Sylva,² James V. Maloney⁴

The Deepwater Horizon blowout is the largest offshore oil spill in history. We present results from a subsurface hydrocarbon survey using an autonomous underwater vehicle and a ship-cabled sampler. Our findings indicate the presence of a continuous plume of oil, more than 35 kilometers in length, at approximately 1100 meters depth that persisted for months without substantial biodegradation. Samples collected from within the plume reveal monoaromatic petroleum hydrocarbon concentrations in excess of 50 micrograms per liter. These data indicate that monoaromatic input to this plume was at least 5500 kilograms per day, which is more than double the total source rate of all natural seeps of the monoaromatic petroleum hydrocarbons in the northern Gulf of Mexico. Dissolved oxygen concentrations suggest that microbial respiration rates within the plume were not appreciably more than 1 micromolar oxygen per day.

The Deepwater Horizon blowout at the MC252 Macondo well site released more than 4 million barrels (636 million liters) of oil into the Gulf of Mexico (1). Its scale and source depth, at 1500 m below the sea surface, represent a relatively uninvestigated category of oil spill. The mechanisms of plume formation are complex due to many factors, including the interplay of gas and oil in multiphase flow, preferential solubility of each oil constituent, and potential gas hydrate formation (2). Consequently, deep-water oil spills are difficult to model, and plume dynamics remain challenging to predict (2–4). Many deep-water models include the Gulf of Mexico in their spill scenarios (4–6).

We initially observed a subsurface layer of oil between 1030- and 1300-m depth during a U.S. Coast Guard-authorized flow-assessment effort at the well site in late May and early June 2010 (fig. S1). To further characterize any resultant plume stemming from the Deepwater Horizon blowout, we performed a 10-day subsurface sampling effort, including three long-range surveys from 19 to 28 June 2010 using the National Deep Submergence Facility's autonomous underwater vehicle (AUV) *Sentry* (fig. S7) and a cable-lowered sample-collection rosette (fig. S2), each

equipped with a *TETHYS* in situ membrane inlet mass spectrometer (7, 8). *Sentry* was chosen for these operations based on this vehicle class's demonstrated utility in characterizing deep-ocean hydrothermal vents (9) and cold seeps (10). Sampling made use of an iterative approach of in situ sensing and automated data analysis to identify select petroleum hydrocarbons and any associated oxygen anomalies. The three *Sentry* surveys, all conducted between 23 and 27 June 2010 at depths in excess of 1000 m, operated for 64 hours to cover a linear distance of 235 km. During these deployments, *Sentry*'s mass spectrometer recorded more than 3500 discrete sample measurements, simultaneously tracking 10 independent chemical parameters in real time. Another 2300 sample measurements were recorded by mass spectrometry during rosette profiling. These mass spectrometers have previously been used for analyzing naturally occurring oil seeps off the coast of California and the Gulf of Mexico (11, 12), tracking subsurface oil leaks from blowout preventers damaged by hurricanes in the Gulf of Mexico (13), and mapping deep-ocean hydrates in real time (10).

Mass spectrometric and fluorescence data, recorded during vertical profiling with the ship's sampling rosette approximately 4 km from the leak source, confirmed the presence of a large plume at ~1000- to 1200-m depth, as well as a more diffuse plume existing between 50- and 500-m depth (Fig. 1). We operationally define a plume as a discrete spatial interval with hydrocarbon signals or signal surrogates (i.e., colored, dissolved organic matter or aromatic hydrocarbon fluorescence) more than two standard deviations above the root mean square baseline variability.

Mass spectra indicate a heterogeneous hydrocarbon mixture changing in composition as a function of depth (Fig. 2); for example, ion peaks

¹Applied Ocean Physics and Engineering Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA.

²Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA.

³Australian Centre for Field Robotics, University of Sydney, Sydney, New South Wales 2006, Australia. ⁴Monitor Instruments Company, Cheswick, PA 15024, USA.

*To whom correspondence should be addressed. E-mail: rcamilli@whoi.edu

associated with aromatic hydrocarbons such as benzene and toluene [i.e., mass/charge ratio (m/z) of 78 and 91, respectively] are present at 1160 m, but greatly attenuated at 10-m depth. This difference in composition may be the result of ventilation to the atmosphere or preferential solubility during upward hydrocarbon migration through the water column. These data suggest that the aromatic hydrocarbons, often associated with adverse biological effects, may be in greater abundance at depth. The sharp decrease of methane and light volatile hydrocarbon fractions in the interval between 30-m depth and the surface implies substantial rates of loss to the atmosphere.

The subsurface plume's lateral direction was then constrained via a continuous rosette casting technique at a constant radius of ~ 5 km from the well site in a circular arc spanning more than 300° (except in the north-northeast direction, due to surface oil collection activity and poor air quality). Based on mass spectra and aromatic fluorometer data, the strongest hydrocarbon readings were encountered at ~ 1100 -m depth, west-southwest of the well site, and a weaker signal was detected northeast of the well site (Fig. 3A).

The first long-range *Sentry* survey—conducted as an east-northeast radial projection from the source at three separate depth intervals (1000, 1150, and 1300 m)—did not encounter petroleum hydrocarbons significantly above background levels. The second survey, carried out as a constant, 1120-m depth “zig-zag” pattern southwest of the leak source, reported elevated petroleum hydrocarbons from its mass spectrometer on each segment of the survey (fig. S8). The third survey extended this pattern further to the southwest. Approximately 27 km from the source, petroleum hydrocarbon values rapidly diminished at this 1120-m survey depth. With the use of its dynamic retasking capability, *Sentry* executed track lines at differing depths until it identified a hydrocarbon maximum at 1160 m. The dive track continued at this modified depth until the survey's conclusion at 35 km from the source. Further AUV operations were discontinued because of deteriorating sea state generated by Hurricane Alex.

The second and third *Sentry* dives transited across horizontal plume anomalies during each of the mission track lines. These data, combined with the rosette-profiling data, indicate a continuous, neutrally buoyant plume as high as 200 m and, in certain areas, more than 2 km in width, moving along a southwestern trend for a distance of more than 35 km from its source (Fig. 3A). Water-velocity data gathered by *Sentry*'s Doppler velocity log during dives two and three measured a southwest trending current at 1100-m depth, averaging 7.8 cm s^{-1} at 247° from true north (fig. S9). The plume's horizontal stability and limited cross-sectional increase as a function of distance from the well site suggest Lagrangian transport. Its track is coincident with the water-current direction at this depth, indicative of topographically controlled transport (14) along an isodepth at the continental slope. Mass spectrom-

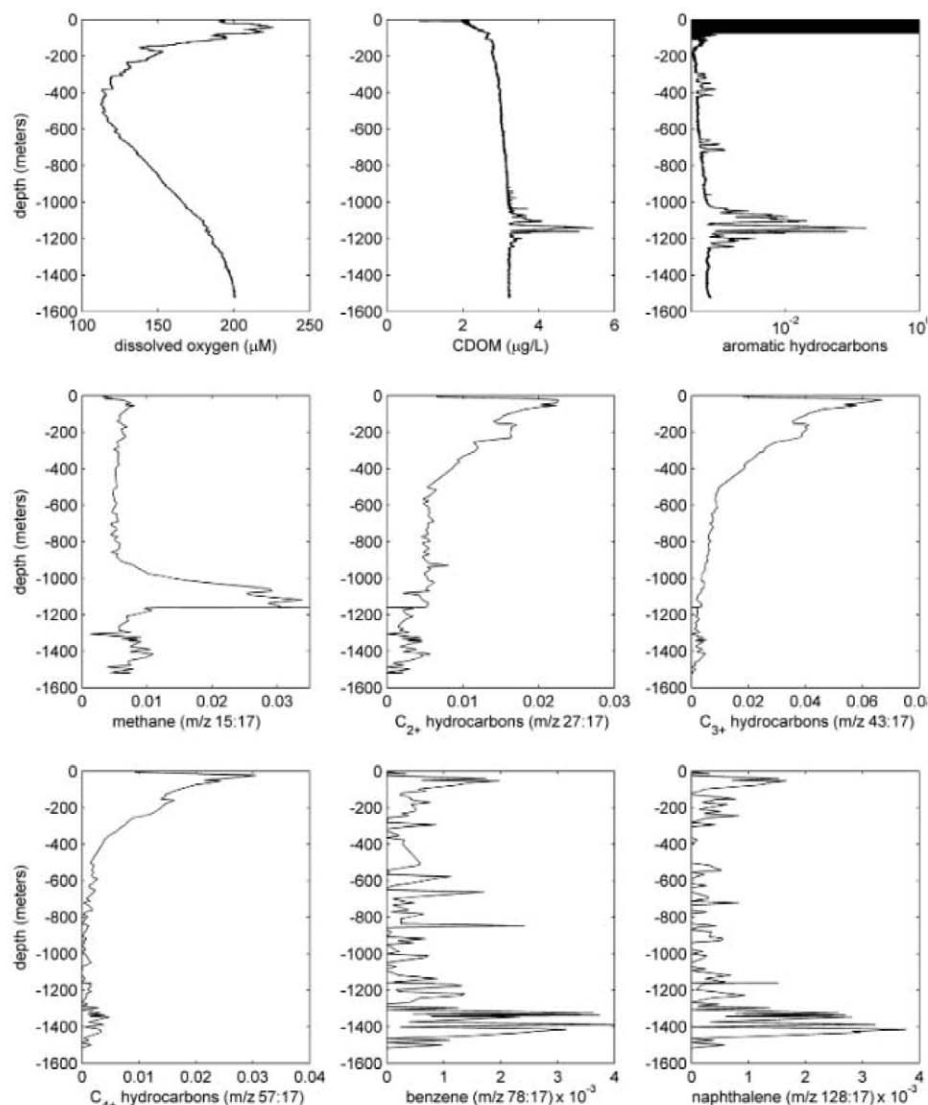


Fig. 1. Vertical profile of water-column chemistry, ~ 4 km from the well site at 28.7352°N 88.3892°W . Aromatic hydrocarbon values are expressed on a relative (logarithmic) scale using in situ ultraviolet fluorometry, whereas hydrocarbon measurements determined via mass spectrometry are ratioed to water (m/z 17) to correct for variability in instrumental response. Mass spectrometer concentration values are unitless (expressed on a relative scale).

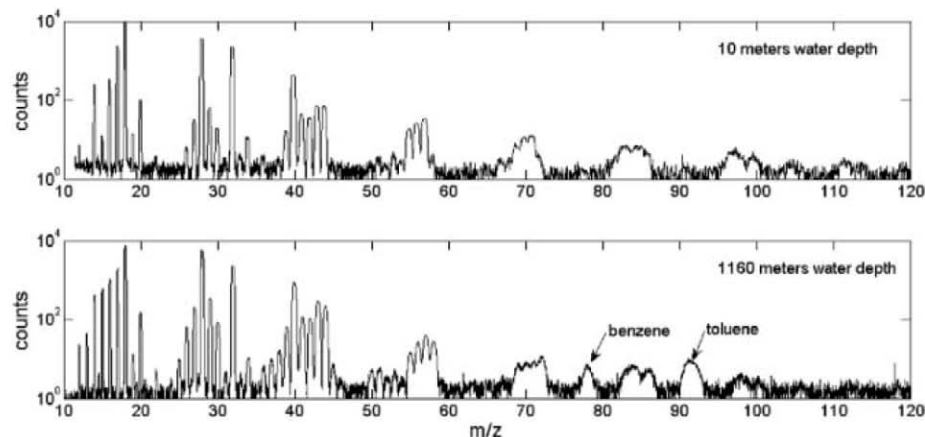


Fig. 2. Mass spectra recorded at 10 and 1160 m during the vertical profile described in Fig. 1.

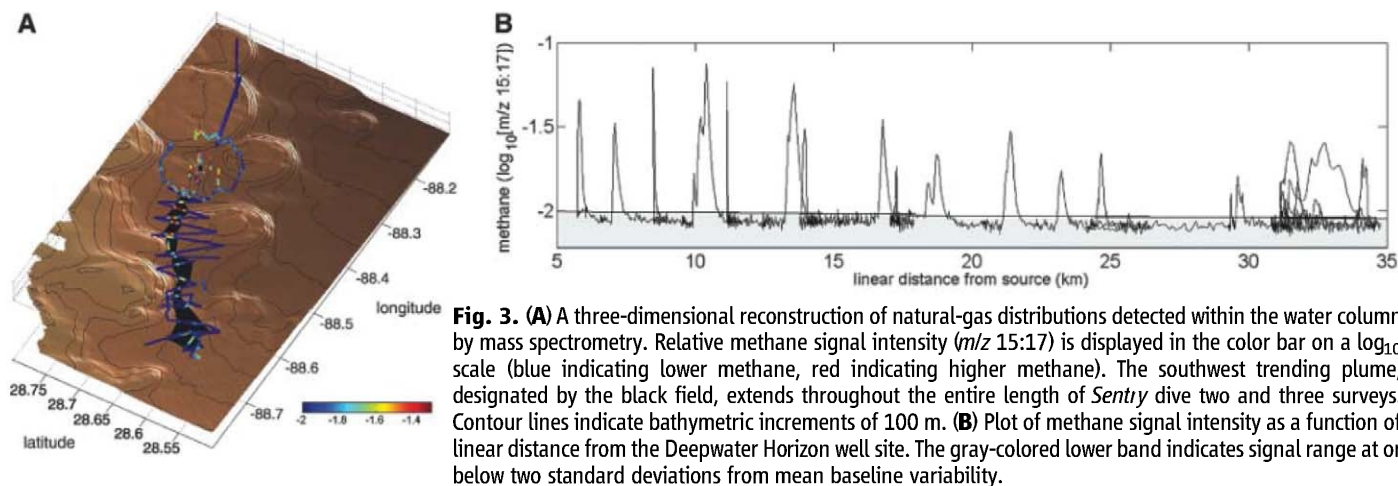
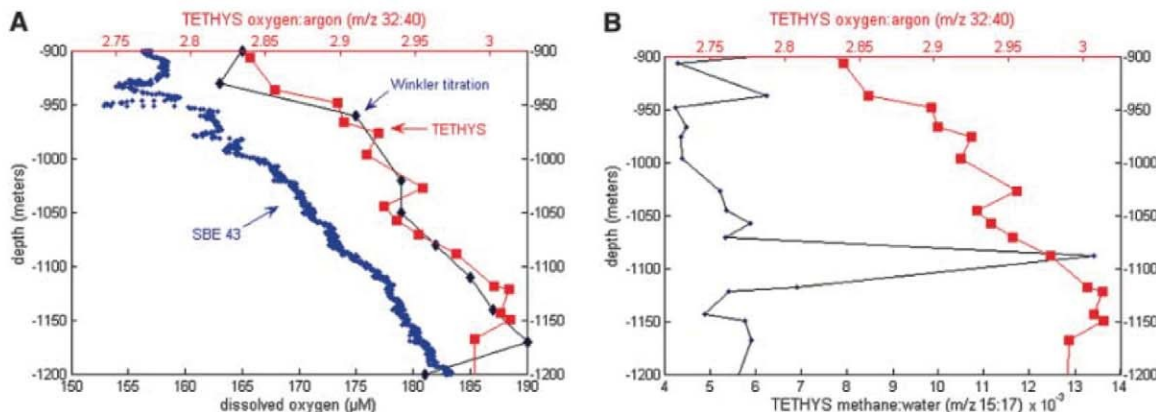


Fig. 4. Vertical profile of water-column chemistry, ~20 km southwest of the well site. (A) Dissolved oxygen estimates based on polarographic electrode (blue diamonds), Winkler titration (black diamonds), and oxygen:argon (red squares). Oxygen and argon have similar solubilities in water. Conservation of argon provides a comparative indicator for relative oxygen saturation. (B) Oxygen:argon ratio relative to methane.



eter indicator ions for dissolved methane exhibited limited amplitude decrease along the plume track during *Sentry* dives, with the local maxima that was encountered at 35 km from the source only 53% less than the maxima encountered at 5.8 km from the source (Fig. 3B). Therefore, it appears likely that the plume extends considerably beyond the survey bounds. Furthermore, the overall decrease in methane maxima and baseline minima as a function of distance from the well site, the similar concentration profiles across all track-line crossings, and the absence of hydrocarbon anomalies at depths below the plume suggest that this horizontal plume is not due to naturally occurring sea-floor petroleum hydrocarbon seeps.

The presence of a southwest trending plume is consistent with findings from an earlier AUV survey conducted by the Monterey Bay Aquarium Research Institute (MBARI) on 2 and 3 June 2010, ~10 km southwest of the well site (15). The body of evidence, including the ROV video that we observed, and the MBARI AUV surveys, along with these *Sentry* and ship rosette surveys, indicate that this plume persisted at this depth interval for months.

Gas chromatographic analyses for only monoaromatic hydrocarbons of several water samples gathered using survey guidance confirm benzene, toluene, ethylbenzene, and total xylene (BTEX) concentrations in excess of $50 \mu\text{g liter}^{-1}$ within

the plume at 16 km downrange from the well site (table S2). A cross-sectional distribution of BTEX within the plume can be calculated from BTEX sample concentrations measured during vertical rosette profiling, and the plume's horizontal profile can be measured by the AUV's mass spectrometer (figs. S11 and S12). A volumetric BTEX flux rate can then be estimated by integrating this cross-section concentration distribution across the observed water-column velocity of 7.8 cm s^{-1} .

Our calculations indicate that more than 5500 kg day^{-1} of BTEX [or ~40 barrels (6400 liters) per day of BTEX using a specific density of 0.85] was introduced into this deep-water-column plume. BTEX is ~1% of the total amount of the oil released (16). Given an oil flow from the well of 53,000 to 62,000 barrels per day (8.4 to 9.9 million liters day^{-1}) (1), the leak released 530 to 620 barrels (84,000 to 99,000 liters) of BTEX per day. Thus, 6 to 7% of all of the BTEX leaked from the well was required to support this plume. These calculations reveal that natural oil seeps cannot be the source of this plume, as the combined inputs for all of the northern Gulf of Mexico are ~1400 barrels (220,000 liters) of total oil per day or 14 barrels (2200 liters) of BTEX per day (17). Therefore, even if all of the natural seeps in the Gulf of Mexico were flowing into the plume, it would support less than half of the

BTEX found in the plume. These findings confirm that a mechanism exists for direct hydrocarbon transfer into deep marine ecosystems. Because our analysis focuses on a limited range of hydrocarbons, the total amount of petroleum hydrocarbons in the plume and the full extent of possible risks to marine biota remain uncertain.

Dissolved oxygen concentrations provide estimates for the relative rate of hydrocarbon biodegradation and oxygen drawdown within the plume. Earlier surveys of the area reported preliminary oxygen deficits of as much as 30% (18, 19), although dissolved oxygen estimates at the spill site using microelectrode sensors have been called into question (20). In certain instances during our rosette-survey operations, the oxygen microelectrode reported localized oxygen-minimum layers in regions that were coincident with the plume depth (fig. S4). Winkler oxygen titrations (21, 22) generally did not confirm these large excursions (fig. S6), although, in some instances such as a hydrocarbon layer encountered at a depth of 930 m at a station 20 km to the southwest of the well site, we observed an oxygen drawdown of a few percent (Fig. 4). Nevertheless, the mean Winkler oxygen concentrations at the 1000- to 1200-m depth interval ($187 \pm 7 \mu\text{M}$) were indistinguishable from mean climatological values ($191 \pm 9 \mu\text{M}$) (23). Furthermore, oxygen:argon

ratios recorded by the mass spectrometers during AUV and rosette operations are in close agreement with Winkler data (Fig. 4A), exhibiting no statistically significant correlation between oxygen and hydrocarbon levels (Fig. 4B). Given that the manufacturer of the oxygen microelectrode sensor advises that hydrocarbon contamination can affect its performance (24), we propose that hydrocarbon contamination could explain some of the low oxygen excursions we observed using this sensor.

The lack of systematic oxygen drawdown within the plume suggests that the petroleum hydrocarbons did not fuel appreciable microbial respiration on the temporal scales of our study. Assuming that the aforementioned west-southwest current carried the hydrocarbon-rich layer away from the well site at the measured velocity of $\sim 7.8 \text{ cm s}^{-1}$ ($\sim 6.7 \text{ km d}^{-1}$), the plume at the end of our survey 35 km from the well site was at least 5 days old. Based on the 95% confidence interval of our Winkler oxygen data from the plume layer ($\pm 2 \mu\text{M}$), we estimate that microbial respiration in the plume was not appreciably more than $\sim 0.8 \mu\text{M O}_2$ per day or, based on elemental formula for straight-chain hydrocarbons, $\sim 0.5 \mu\text{M C}$ per day. This suggests that if the hydrocarbons are indeed susceptible to biodegradation, then it may require many months before microbes substantially attenuate the hydrocarbon plume to the point that oxygen-minimum zones develop that are intense enough $\{[\text{O}_2] < 63 \mu\text{M} (25, 26)\}$ to threaten Gulf of Mexico fisheries.

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Deep-Sea Oil Plume Enriches Indigenous Oil-Degrading Bacteria

Terry C. Hazen^{1*}, Eric A. Dubinsky¹, Todd Z. DeSantis¹, Gary L. Andersen¹, Yvette M. Piceno¹, Navjeet Singh¹, Janet K. Jansson¹, Alexander Probst¹, Sharon E. Borglin¹, Julian L. Fortney¹, William T. Stringfellow^{1,2}, Markus Bill¹, Mark E. Conrad¹, Lauren M. Tom¹, Krystle L. Chavarria¹, Thana R. Alusi¹, Regina Lamendella¹, Dominique C. Joyner¹, Chelsea Spier², Jacob Baelum¹, Manfred Auer¹, Marcin L. Zemla¹, Romy Chakraborty¹, Eric L. Sonnenthal¹, Patrik D'haeseleer⁴, Hoi-Ying N. Holman¹, Shariff Osman¹, Zhenmei Lu³, Joy D. Van Nostrand³, Ye Deng³, Jizhong Zhou^{1,3}, Olivia U. Mason¹

The biological effects and expected fate of the vast amount of oil in the Gulf of Mexico from the Deepwater Horizon blowout are unknown owing to the depth and magnitude of this event. Here, we report that the dispersed hydrocarbon plume stimulated deep-sea indigenous γ -Proteobacteria that are closely related to known petroleum degraders. Hydrocarbon-degrading genes coincided with the concentration of various oil contaminants. Changes in hydrocarbon composition with distance from the source and incubation experiments with environmental isolates demonstrated faster-than-expected hydrocarbon biodegradation rates at 5°C. Based on these results, the potential exists for intrinsic bioremediation of the oil plume in the deep-water column without substantial oxygen drawdown.

Assessing the environmental and public health impacts of the Deepwater Horizon blowout is difficult owing to the extreme depth of the blowout and the large volumes of oil

released. Moreover, the effectiveness of the primary initial mitigation strategy (e.g., injecting the oil dispersant Corexit 9500 directly at the well-head in a water depth of 1544 m) is difficult to

assess despite initial analysis of its potential toxicity (1). An optional strategy for remediation of the deep underwater plume is to use the intrinsic bioremediation potential of deep-sea microorganisms to degrade the oil. This strategy depends on a number of environmental factors, including a favorable response of indigenous microorganisms to an increased concentration of hydrocarbons and/or dispersant.

To determine the impact of the deep hydrocarbon plume on the marine microbes residing in the plume and the rates of hydrocarbon biodegradation, we collected deep-water samples from two ships between 25 May 2010 and 2 June 2010. In total, we analyzed the physical, chemical, and microbiological properties (fig. S1) of 17 deep-water samples from across the Gulf of Mexico (2).

¹MS 70A-3317, One Cyclotron Road, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA. ²University of the Pacific, Ecological Engineering Research Program, 3601 Pacific Avenue, Stockton, CA 95211, USA. ³University of Oklahoma, 101 David L. Boren Boulevard, Norman, OK 73072, USA. ⁴Biosciences and Biotechnology Division, Lawrence Livermore National Laboratory, Livermore, CA 94550, USA.

*To whom correspondence should be addressed. E-mail: tchazen@lbl.gov

We detected a deep-sea oil plume from 1099 to 1219 m at distances of up to 10 km from the wellhead (fig. S2). Owing to its composition (fig. S3), the plume was likely dispersed MC252 oil, a conclusion also reached by Camilli *et al.* (3). At most locations where the plume was detected, there was a slight decrease in oxygen concentration indicative of microbial respiration and oxygen consumption, as would be expected if the hydrocarbons were being catabolized (Fig. 1). Oxygen saturation within the plume averaged 59%, while outside the plume it was 67%. Extractable hydrocarbons (e.g., octadecane) ranged from nondetectable in the nonplume samples to 9.21 $\mu\text{g/liter}$ in plume samples (table S1). Volatile aromatic hydrocarbons were significantly higher in the plume interval (mean 139 $\mu\text{g/liter}$) than in

the nonplume samples from similar depths. The average temperature within the plume interval was 4.7°C and the pressure was 1136 dB. Soluble orthophosphate, and total ammonia-N, were detected at similar concentrations within and outside the plume interval (table S1).

The dispersed oil plume affected both microbial cell densities and composition (Fig. 1 and table S1). Cell densities in the plume ($5.51 \pm 0.33 \times 10^4$ cells/ml) were higher than outside the plume ($2.73 \pm 0.05 \times 10^4$ cells/ml). Phospholipid fatty acid analysis also confirmed an increase in microbial biomass in the plume (0.57 pmol lipids/ml) versus outside the plume (0.23 pmol lipids/ml) (table S1). In addition to the observed increase in cell densities, PhyloChip 16S ribosomal RNA (rRNA) microarray analysis (fig. S4) suggests

that the plume significantly altered the microbial community composition and structure. Ordination of bacterial and archaeal 16S rRNA gene composition revealed two distinct clusters of samples: one composed entirely of plume samples with detected oil and the other of nonplume samples (Fig. 2). No physical or chemical factors other than hydrocarbons and nitrates were significantly different between these groups (table S1), indicating that microorganisms were responding directly to the presence of dispersed oil.

In plume samples, PhyloChip analysis revealed that 951 distinct bacterial taxa in 62 phyla were present (fig. S4), but only 16 distinct taxa that were all classified as γ -Proteobacteria were significantly enriched in the plume relative to nonplume samples (table S2 and fig. S5). Nearly all

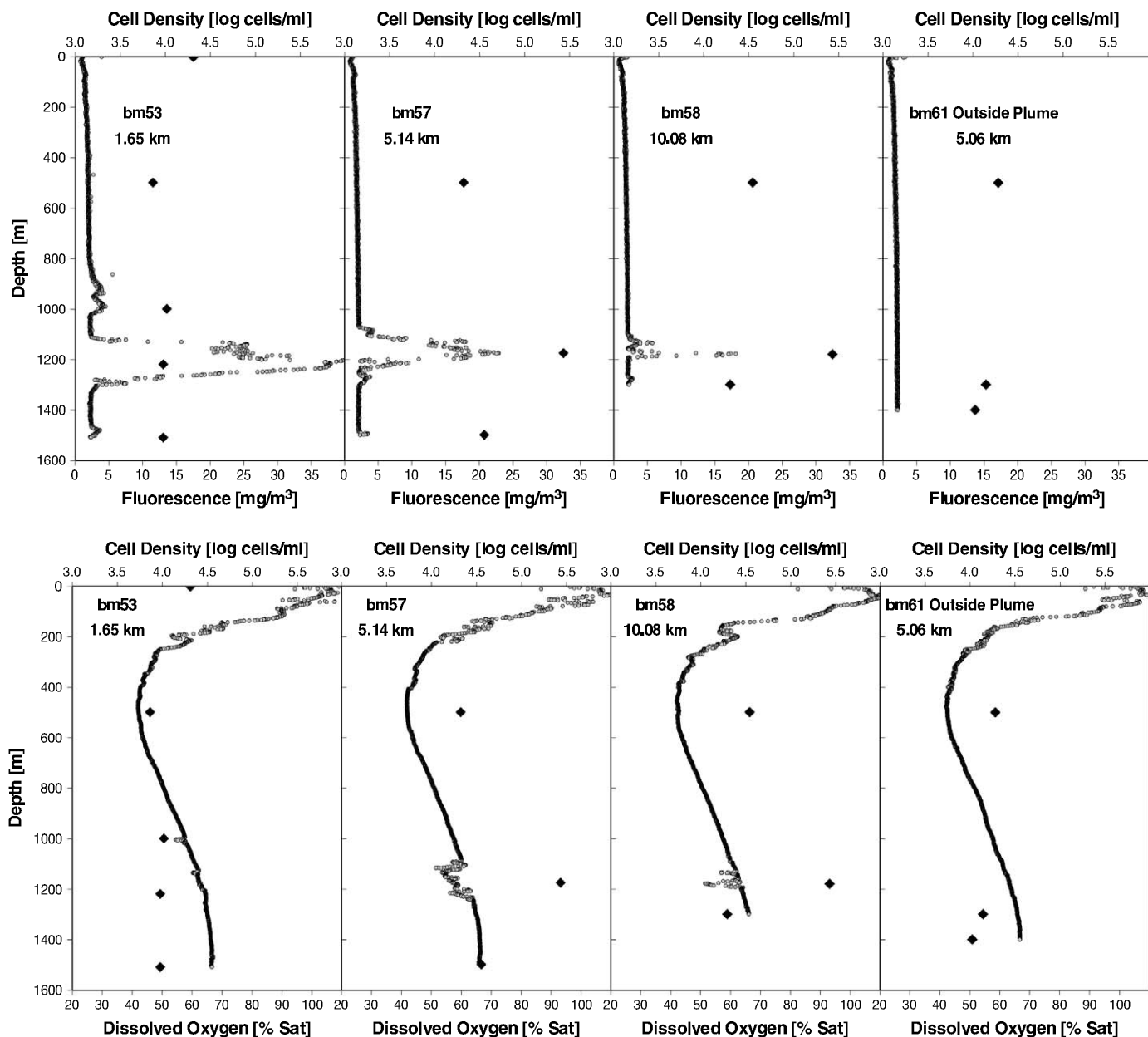


Fig. 1. Characteristic depth profiles of cell density, fluorescence, and dissolved oxygen for distances from the source (BM53, BM57, and BM58) and one nonplume site (BM61). Diamonds indicate cell density.

Fig. 2. Microbial community analysis of deep-water plume and nonplume samples. Differences in composition of (A) 16S rRNA gene sequences measured by PhyloChip and (B) phospholipid fatty acids were analyzed with nonmetric multidimensional scaling ordination of Bray-Curtis distances (stress = 3.98 and 4.55, respectively). Plume and nonplume communities were significantly different as determined by permutational analysis of variance ($P = 0.005$ for both) and delineated with lines for clarity.

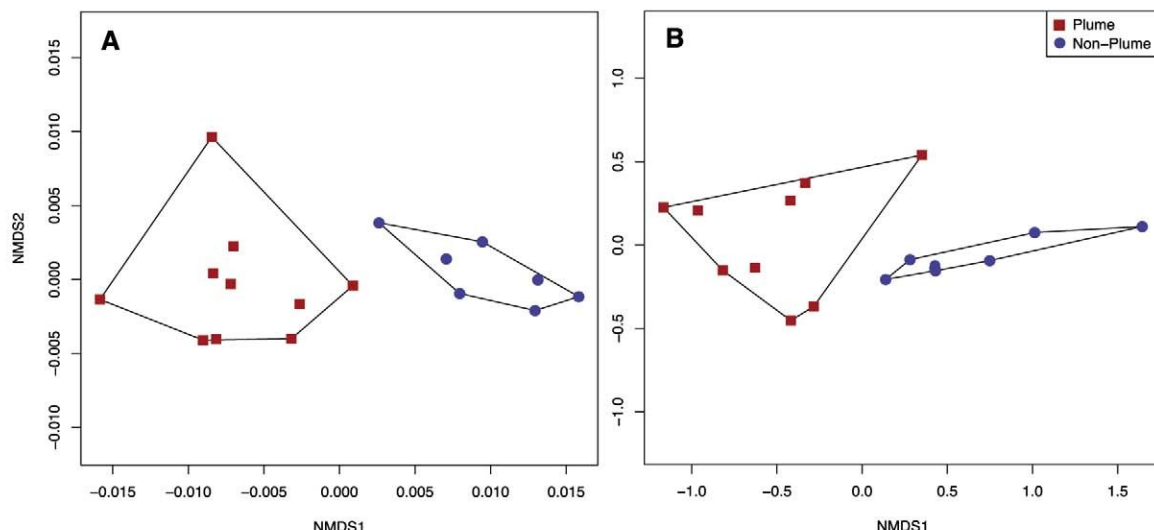
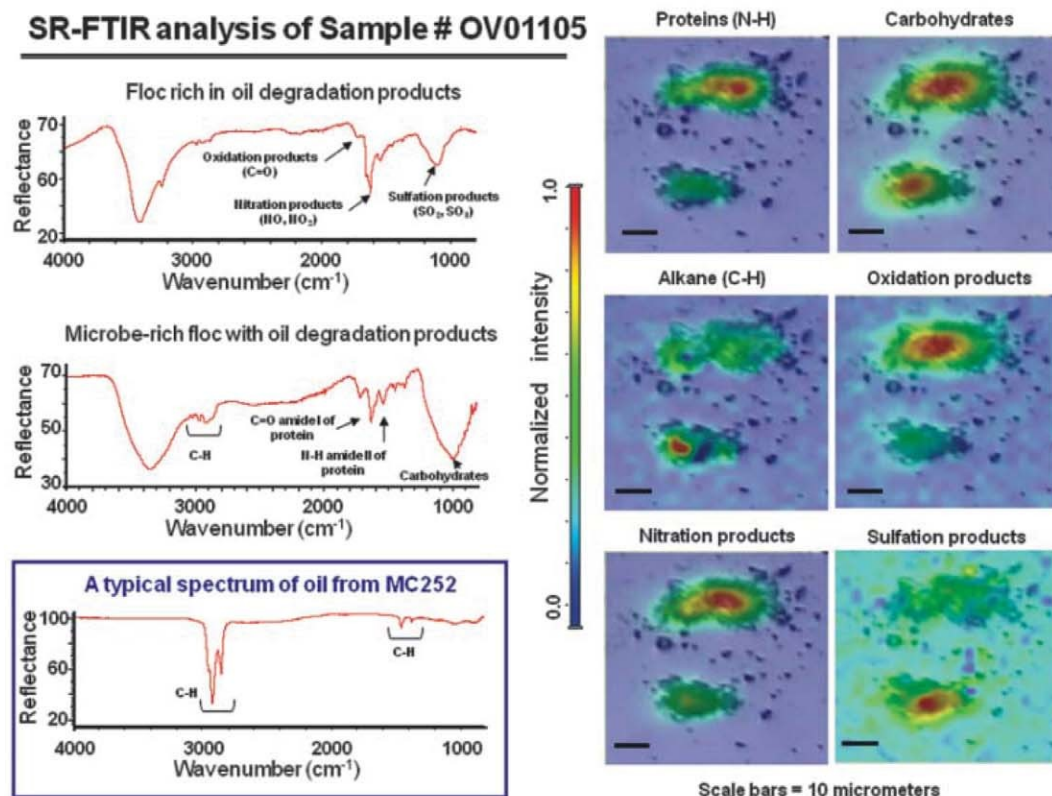


Fig. 3. SR-FTIR images ($\sim 60 \mu\text{m}$ by $60 \mu\text{m}$) showing the distribution of microorganisms, oil, and oil degradation products in a "floc." Distribution heat map of the protein amide II vibration modes at $\sim 1542 \text{ cm}^{-1}$ and the carbohydrates vibration modes at $\sim 1000 \text{ cm}^{-1}$ (20). Distribution heat map of alkane C-H vibration modes in oil from MC252. Distribution heat map of carbonyl (C=O) vibration modes at $\sim 1730 \text{ cm}^{-1}$ in oil oxidation products, of nitrogen oxides vibration modes at $\sim 1610 \text{ cm}^{-1}$ in nitration products, and of sulfur oxides vibration modes at $\sim 1150 \text{ cm}^{-1}$ in sulfation products. Scale bars: $10 \mu\text{m}$. Reflectance is given in percentage units.



of these enriched taxa have representatives that degrade hydrocarbons or are stimulated by the presence of oil in cold environments (table S2). Plume-enriched bacteria include many psychrophilic and psychrotolerant species that have been observed in low-temperature marine environments (table S2) (4–6). Although cell densities are higher in the plume, taxonomic richness was lower and the diversity of enriched bacteria was restricted to a few γ -Proteobacteria.

Cloning and sequencing revealed that deep-sea plume samples from station BM58 ($\sim 10.08 \text{ km}$ from the MC252 wellhead) and station OV011

($\sim 1.5 \text{ km}$ from the wellhead) were dominated by the order *Oceanospirillales* in the γ -Proteobacteria. More than 90% of all sequences in both plume samples (10 km between sampling stations) belonged to a single operational taxonomic unit (OTU) that is most closely related to *Oceanospirillales* (Fig. 3). In a control sample (site OV003) collected 39.1 km southwest of the wellhead, this same OTU represented only 5% of all sequences analyzed (Fig. 3). In addition, this taxon was detected in all 10 oil plume samples analyzed by the PhyloChip and was significantly enriched relative to background deep seawater with no oil

(table S1). The cultured representatives most closely related to the OTU in plume samples were *Spongiispira norvegica* (95% similar) and *Oceaniserpentilla haliotidis* (94% similar). The observed sequences in the plume samples form a clade with two distinct *Oceanospirillales* groups. One of these groups is largely composed of known psychrophilic hydrocarbon degraders and microorganisms from hydrocarbon-dominated environments (5, 7, 8), including *Oleispira antarctica*, *Thalassolituus oleivorans*, and *Oleiphilus messinensis* (fig. S5).

The three dominant phospholipid fatty acids detected in the plume samples were C16:0,

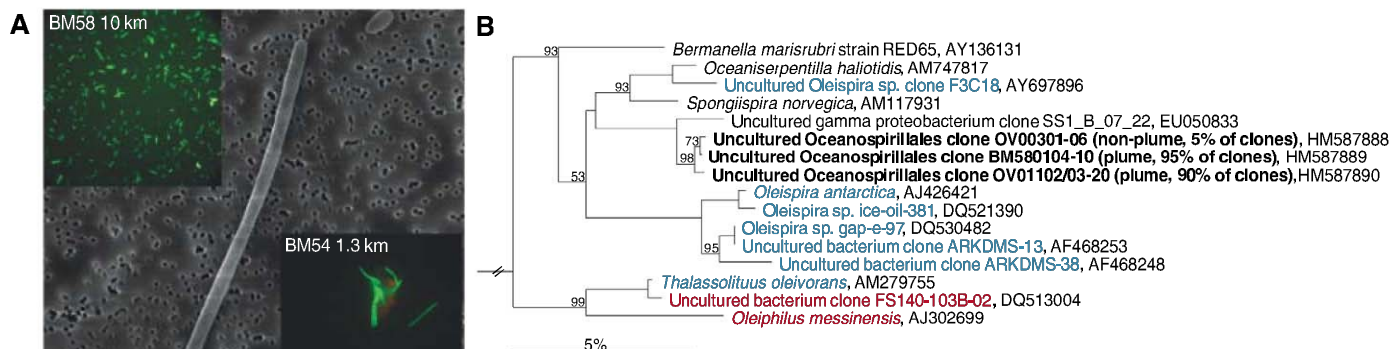


Fig. 4. (A) Dominant bacteria at 1099 to 1219 m (scanning electron micrograph) and acridine orange stain (inset) with distance from source. **(B)** Neighbor-joining tree showing the phylogenetic relationships of the dominant bacterium in deep-sea plume samples. Relative abundance of the dominant bacterium was 90 to 95% of plume samples and 5% of the nonplume sample (shown in parentheses). Psychrophilic, hydrocarbon-degrading bacteria, as well as uncultured organisms from low-temperature, hydrocarbon-dominated environments, are shown in blue. Organisms shown in red are either known hydrocarbon degraders or are from hydrocarbon-dominated ecosystems but are not from low-temperature environments. Bootstrap values based on 1000 replicates of $\geq 50\%$ are shown at branch points. *Aquifex pyrophilus* (GenBank accession M83548) was used as the outgroup.

C16:1w7c, and C18:1w9c (table S3), which have been reported as the dominant lipids in the *Oleispira antarctica*, in some strains of the *Oceaniserpentilla haliotis* (4), and in a consortium of marine hydrocarbon-degrading bacteria (9). 18:1w9/c ratios that have been reported to increase in oil-contaminated environments (10, 11) were slightly elevated in plume samples (average 0.21) compared to nonplume samples (average 0.14) but were not strongly correlated with oil concentrations (table S1). Multivariate analysis of phospholipid fatty acid (PLFA) profiles from each sample revealed distinct clustering of plume and nonplume samples similar to community analysis of microarray data (Fig. 2).

Microscopic examination of cells collected within the plume also revealed that the dominant cell type exhibits a distinctive morphology typical of the *Oceanospirillales* (Fig. 4). Total bacterial densities were also significantly correlated with MC252 alkane concentration in the plume (fig. S5). Synchrotron radiation-based Fourier-transform infrared (SR-FTIR) spectromicroscopy revealed absorptions at ~ 1730 , ~ 1610 , and ~ 1150 cm^{-1} that are associated with biomolecule-rich regions of a cellular floc (Fig. 3). These absorption features are well described for the carbonyl (C=O), nitrogen oxides, and sulfur oxides vibration modes (12) and are characteristic of oil degradation products (13). These SR-FTIR spectra are not consistent with those typically found in marine macroaggregates (14), nor are they consistent with nonplume samples at the same depth.

To understand the distribution of oil-degrading genes within the plume, we analyzed five samples (BM053, BM054, BM057, BM058, BM064) from the MC252 dispersed oil plume as well as five uncontaminated, control samples (OV003, OV004, OV009, OV013 and OV014) collected from plume depth with GeoChip functional array (table S4) (15, 16). Altogether, 4000 to 5000 functional genes were detected per sample, among which 1652 genes are involved in hydrocarbon degradation. Detrended correspondence analysis

showed that microbial community functional composition and structure were considerably different between oil-plume and nonplume control samples (fig. S7), which is consistent with PhyloChip analysis. Many of the genes involved in hydrocarbon degradation were significantly ($P < 0.05$ or 0.01) increased in oil plume samples (figs. S8 and S9). Statistical analysis by Mantel test showed that the overall microbial functional composition and structure were significantly correlated with many key oil contaminants, including isopropylbenzene, *n*-propylbenzene, *tert*-butylbenzene, 1,2,4-trimethylbenzene, *p*-isopropyltoluene, *n*-butylbenzene, and naphthalene (table S5). Analysis based on individual genes showed that the changes of many hydrocarbon degradation genes are significantly correlated with the concentrations of oil contaminants (table S6). For instance, the *phdCI* gene encoding carboxylate isomerase for naphthalene degradation correlates with several hydrocarbons (table S6). These results indicated that a variety of hydrocarbon-degrading populations exist in the deep-sea plume and that the microbial communities appear to be undergoing rapid dynamic adaptation in response to oil contamination. These results also imply that there exists a potential for intrinsic bioremediation of oil contaminants in the deep sea and that oil-degrading communities could play an important role in controlling the ultimate fates of hydrocarbons in the Gulf.

The bioremediation potential largely depends on the rates of biodegradation in the plume. We calculated maximum biodegradation rates using two data sets from the field and two from laboratory microcosms representing concentrations of C13 to C26 *n*-alkanes (table S7). The degradation rate coefficients and half-life values (table S7 and figs. S10 and S11), calculated from the alkane data from these four sources with the first-order rate equation (10, 17), are similar to those reported for comparable temperature and field conditions (10, 17–19). Despite the varying field and microcosm conditions, the oil half-lives are 1.2 to 6.1 days (table S7). The field half-lives

should in part reflect the effect of mixing and dilution, but the similarity of the rate of disappearance of alkanes in the plume to the rates observed in the laboratory suggest it is possible that the actual degradation of alkanes lies within this range. The possibility that biodegradation largely controls the disappearance of alkanes is also supported by the preferential degradation of short-chain alkanes, as represented in the increase in the ratio of C26/C15 alkanes over 10 km, from less than 1 to more than 3 (fig. S18). For each data set, decay constants were similar for all alkanes measured in all samples, with the exception of the plume samples from the nonlipid fraction collected on 0.2- μm filters. Because these results represent extraction from free-phase oil or oil absorbed to the membrane filter, it is likely that the higher rates seen for the shorter-chain alkanes are due to additional losses in collected sample resulting from dissolution into seawater; however, there is a correlation of longer-chain alkane concentration with cell densities in the plume (fig. S6). The oil biodegradation rates reported here at 5°C are explained partly by the relatively light nature of this crude (which contains a large volatile component that is more readily degraded), the dispersed nature of the deep plume (small oil particle size), the low overall concentrations of oil in the deep plume, and the frequent episodic oil leaks from natural seeps in this area that the deep-sea microbial community may have adapted to over long periods of time.

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R/V *Brooks McCall*. The National Center for Biotechnology Information accession numbers of the 16S rRNA genes retrieved from clone library analyses are HM587888, HM587889, and HM587890. Sequences for 16S rRNA are also available at greengenes.lbl.gov.

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1195979/DC1
Methods

Figs. S1 to S18

Tables S1 to S8

References

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Propane Respiration Jump-Starts Microbial Response to a Deep Oil Spill

David L. Valentine,^{1*} John D. Kessler,² Molly C. Redmond,¹ Stephanie D. Mendes,¹ Monica B. Heintz,¹ Christopher Farwell,¹ Lei Hu,² Franklin S. Kinnaman,¹ Shari Yvon-Lewis,² Mengran Du,² Eric W. Chan,² Fenix Garcia Tigreros,² Christie J. Villanueva¹

The Deepwater Horizon event resulted in suspension of oil in the Gulf of Mexico water column because the leakage occurred at great depth. The distribution and fate of other abundant hydrocarbon constituents, such as natural gases, are also important in determining the impact of the leakage but are not yet well understood. From 11 to 21 June 2010, we investigated dissolved hydrocarbon gases at depth using chemical and isotopic surveys and on-site biodegradation studies. Propane and ethane were the primary drivers of microbial respiration, accounting for up to 70% of the observed oxygen depletion in fresh plumes. Propane and ethane trapped in the deep water may therefore promote rapid hydrocarbon respiration by low-diversity bacterial blooms, priming bacterial populations for degradation of other hydrocarbons in the aging plume.

The oil leakage following the sinking of the Deepwater Horizon in the Gulf of Mexico was unprecedented because it occurred at 1.5-km water depth. The slow buoyant migration of petroleum from this depth allows time for dissolution of volatile hydrocarbons (1–3), including the natural gases methane (CH₄), ethane (C₂H₆), propane (C₃H₈), and butane (C₄H₁₀), that would readily escape to the atmosphere if released in shallow water. The resulting plumes of dissolved gas may co-occur with oil in the water (3) or may occur without oil because of gas fractionation processes during ascent (4). Based on the cumulative discharge estimates through 1 August 2010 (5) and a gas-to-oil ratio of 3000 ft³ barrel⁻¹ (at atmospheric pressure), 1.5 × 10¹⁰ moles of natural gas were potentially emitted to the deep water over the course of the spill, in addition to the oil (6). We investigated the distribution, fate, and impacts of these hydrocarbons at 31 stations located 1 to 12.5 km from the active spill site (Fig. 1A) during the PLUMES (Persistent and Localized Underwater Methane Emission Study)

expedition of the research vessel *Cape Hatteras*, 11 to 21 June 2010 (6).

In the vicinity of the leaking well, propane, ethane, and methane were most abundant at depths greater than 799 m and formed plume structures (Fig. 1C and figs. S1 to S3) with dissolved concentrations as high as 8 μM, 16 μM, and 180 μM for the three gases, respectively. These gases were orders of magnitude less concentrated at shallower depths, confirming suggestions (7), results from a noncalibrated spectrometric survey (3), and models (1, 2) that the majority of the emanated gas dissolves or is otherwise partitioned (e.g., as gas hydrate) at depth and remains there. We defined a hydrocarbon plume by a methane concentration >500 nM, which is roughly 20 to 50 times as high as background levels of methane in the Gulf of Mexico (8) and is above the methane levels typically found around natural seeps (9–13). We observed deep (>799 m) hydrocarbon plumes at 29 of the 31 stations where methane measurements were made. One persistent plume at 1000- to 1200-m depth located to the southwest of the spill site (Fig. 1C) was identified previously (3, 14–16). We also identified separate plumes at similar depths to the north and to the east, as well as a distinctive shallower plume at 800- to 1000-m depth also located to the east (figs. S2 and S3). The multiple plumes in opposing directions presumably originated at different

times and indicate complex current patterns in the area before sampling.

The ratio of methane to ethane and propane varied substantially throughout the deep plumes. At the locations with highest hydrocarbon concentrations, the lower end-member values approached 10.85 for CH₄/C₂H₆ (Fig. 2A) and 19.8 for CH₄/C₃H₈ (Fig. 2B) and could therefore represent the gas ratios at the plume origin. Numerous locations display higher ratios, which we interpret as preferential loss of propane and ethane relative to methane, a pattern reported previously for biodegradation in hydrocarbon seeps (17). Variation in the C₂H₆/C₃H₈ ratio (Fig. 2C) further suggests preferential loss of propane compared with ethane, also an established biodegradation pattern (17). Methane's conservative behavior is supported by generally slow rates of oxidation, as measured with a tritium tracer approach (table S1).

Because bacterial propane, ethane, and methane consumption occurs with characteristic kinetic isotope effects (17), we measured the carbon isotopic composition of these gases in deep plume waters to assess the extent of their biodegradation. Samples with CH₄/C₃H₈ > 19.8 displayed a relative ¹³C-enrichment in propane. Comparison of the ¹³C-propane enrichment to the fractional loss of propane (Fig. 2D), determined from the CH₄/C₃H₈ ratio, indicates that biodegradation occurs (18, 19) with an isotopic enrichment factor (ε) of −6.3. The value of ε for ethane (−11.8) based on CH₄/C₂H₆ ratios also suggests that biodegradation is occurring. Both values are similar to the minimum respective values of −5.9 and −11.2 determined from a previous mesocosm study (17). A lack of notable ¹³C enrichment for methane [δ¹³C-CH₄ = −61.1 ± 2.2‰ (per mil); *n* = 18] is further evidence of its conservative behavior in the fresh plumes.

To assess the importance of ethane and propane as aerobic respiratory substrates, we compared their loss patterns with observed oxygen anomalies in the deep-water column (Fig. 1B). Oxygen levels, measured in situ with an oxygen sensor and confirmed onboard ship through Winkler titrations, systematically declined in the plume horizon (Fig. 1D and fig. S4). Regression of the observed oxygen anomaly against the propane anomaly (6) indicates that 58% of the oxygen anomaly can be linked to propane (Fig. 3A).

¹Department of Earth Science and Marine Science Institute, University of California, Santa Barbara, CA 93106, USA. ²Department of Oceanography, Texas A&M University, College Station, TX 77843–3146, USA.

*To whom correspondence should be addressed. E-mail: valentine@geol.ucsb.edu

A similar analysis for propane plus ethane indicates that 70% of the oxygen anomaly can be linked to respiration of these two gases together (Fig. 3A). Therefore, ethane and propane are dominant respiratory substrates during the early development of deep-water hydrocarbon plumes. This relationship may break down as plumes mature because propane and ethane are removed before methane (Fig. 2, A and B) and likely

before less soluble n-alkanes greater than five carbons in length. Once ethane and propane have been consumed, respiration rates are expected to drop; such a drop, when combined with mixing, could account for the weak respiration signal ($<0.8 \mu\text{M d}^{-1}$) reported for the more distal southwest plume horizon by other investigators (3). The residual oxygen anomaly not accounted for by propane and ethane respiration ($\sim 30\%$ plus

a biomass correction) presumably derives from other hydrocarbons. Butane, which is also readily biodegraded (17, 20), is likely a substantial contributor, as it constituted at least 0.75% of the dissolved gas in the freshest plume samples but was low in concentration in more biodegraded samples (table S1).

We investigated the bacterial capacity for propane and ethane biodegradation by adding

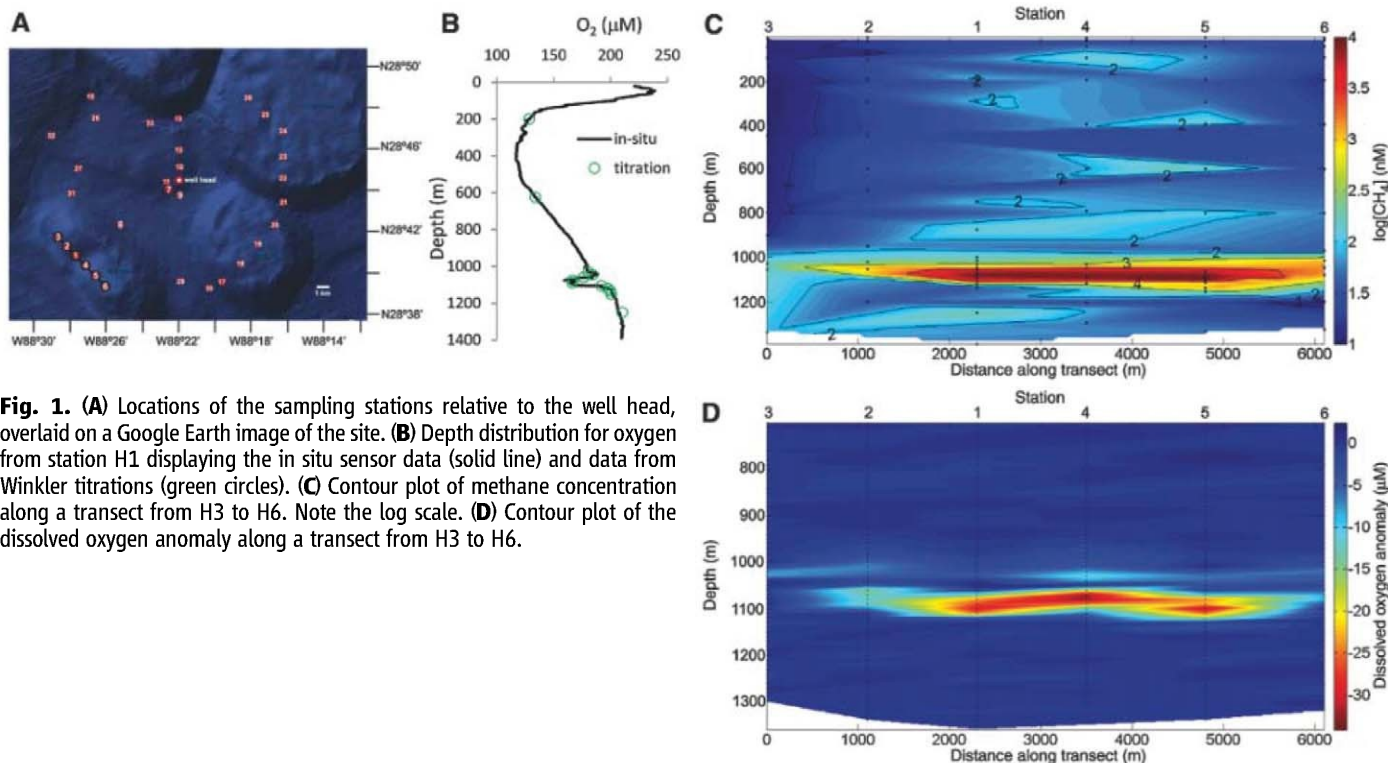


Fig. 1. (A) Locations of the sampling stations relative to the well head, overlaid on a Google Earth image of the site. (B) Depth distribution for oxygen from station H1 displaying the in situ sensor data (solid line) and data from Winkler titrations (green circles). (C) Contour plot of methane concentration along a transect from H3 to H6. Note the log scale. (D) Contour plot of the dissolved oxygen anomaly along a transect from H3 to H6.

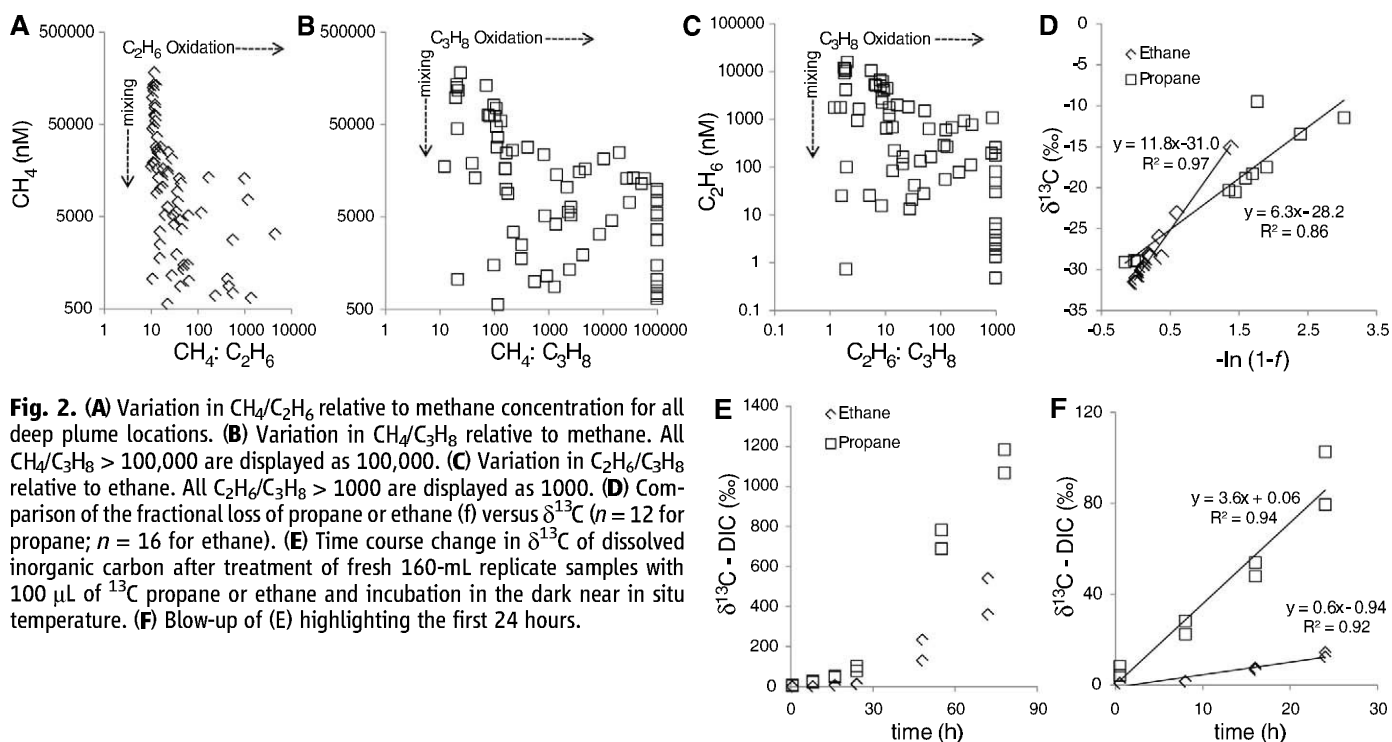


Fig. 2. (A) Variation in $\text{CH}_4/\text{C}_2\text{H}_6$ relative to methane concentration for all deep plume locations. (B) Variation in $\text{CH}_4/\text{C}_3\text{H}_8$ relative to methane. All $\text{CH}_4/\text{C}_3\text{H}_8 > 100,000$ are displayed as 100,000. (C) Variation in $\text{C}_2\text{H}_6/\text{C}_3\text{H}_8$ relative to ethane. All $\text{C}_2\text{H}_6/\text{C}_3\text{H}_8 > 1000$ are displayed as 1000. (D) Comparison of the fractional loss of propane or ethane (f) versus $\delta^{13}\text{C}$ ($n = 12$ for propane; $n = 16$ for ethane). (E) Time course change in $\delta^{13}\text{C}$ of dissolved inorganic carbon after treatment of fresh 160-mL replicate samples with 100 μL of ^{13}C propane or ethane and incubation in the dark near in situ temperature. (F) Blow-up of (E) highlighting the first 24 hours.

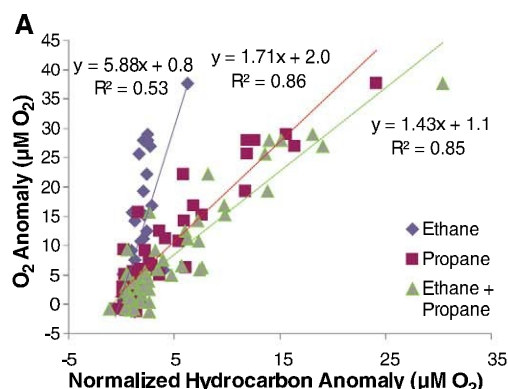
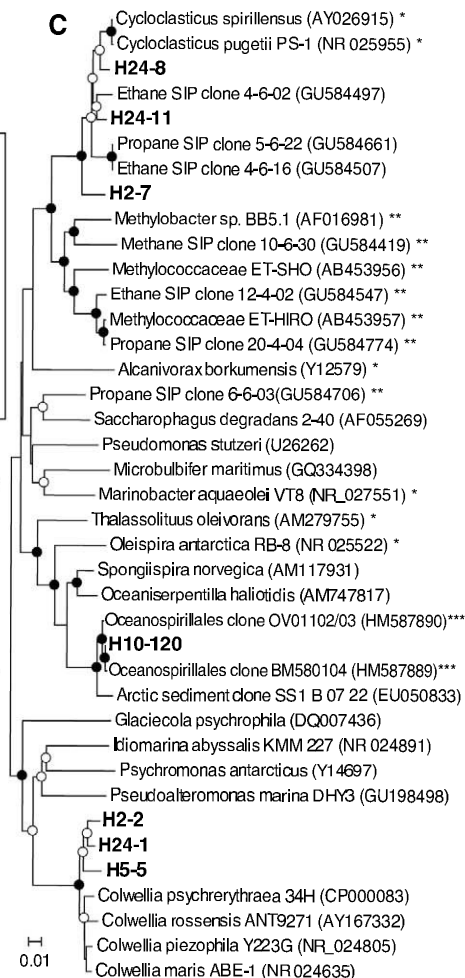
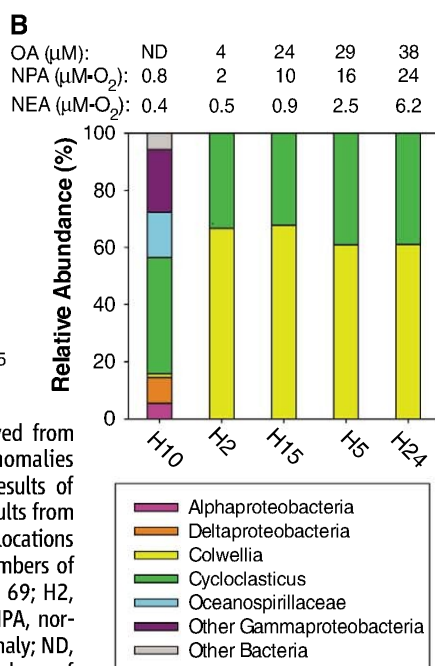


Fig. 3. (A) Comparison of the oxygen anomaly derived from Winkler titrations with normalized hydrocarbon anomalies derived from variation in $\text{CH}_4/\text{C}_2\text{H}_6$ and $\text{CH}_4/\text{C}_3\text{H}_8$. Results of linear regression are provided ($n = 36$ for each). **(B)** Results from DNA surveys for bacterial 16S rRNA genes at five plume locations representing different levels of biodegradation. The numbers of clones sequenced for each location are as follows: H10, 69; H2, 36; H15, 59; H5, 36; H24, 44. OA, oxygen anomaly; NPA, normalized propane anomaly; NEA, normalized ethane anomaly; ND, not detected. **(C)** A phylogenetic tree displaying the relatedness of *Gammaproteobacteria* identified in this study (bold), and selected relatives. *, oil degrader; **, methane, ethane, or propane oxidizer; ***, sequence from (16).

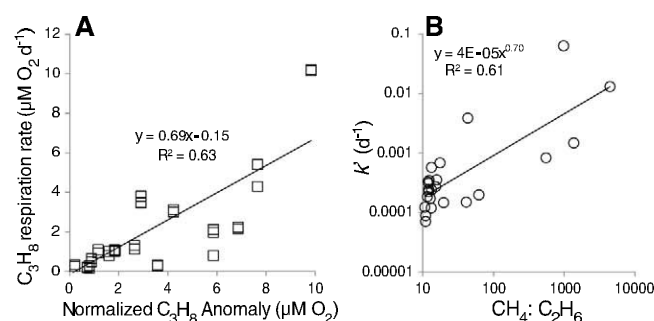


^{13}C -labeled substrate into freshly collected plume waters and monitoring label conversion to ^{13}C - CO_2 . Time series measurements conducted at station H1 (Fig. 2, E and F) reveal an initial stage when product accumulates at a constant rate (Fig. 2F), followed by a marked increase after 24 hours (Fig. 2E). We interpret the initial rate as the maximum potential rate of biodegradation by the basal population, with saturation of the population's enzymatic capacity leading to zero-order kinetic behavior. The later increase then indicates a growth or biosynthetic response by the microbial community to the elevated substrate level. This interpretation is supported by the observation that zero-order kinetic behavior occurs at high levels of added label, while higher-order kinetic behavior seems to result from addition of smaller quantities of labeled substrate (fig. S5). Samples treated with mercuric chloride showed no appreciable production of ^{13}C - CO_2 , further confirming the biological nature of ethane and propane oxidation.

Variations in consumption of propane and methane by the developing microbial community were assessed for different oxygen anomalies using ^{13}C and ^3H tracers, respectively. In all cases, fresh duplicate plume samples were incubated in the dark near in situ temperature with tracer for 24 hours. In methane measurements, ^3H - CH_4

Fig. 4. (A) Comparison of potential propane oxidation rates measured by ^{13}C tracer conversion, with propane anomalies determined from the $\text{CH}_4/\text{C}_3\text{H}_8$ ratios in the source water. $n = 14$. **(B)** Comparison of the effective pseudo first-order rate constant (k') for methane oxidation versus the extent of ethane loss relative to methane ($\text{CH}_4/\text{C}_2\text{H}_6$). $n = 23$.

tracer levels were $<2\%$ of ambient methane, allowing for a direct calculation of methane oxidation (13). In propane measurements, the lower sensitivity for stable isotope analyses necessitated addition of large quantities of tracer, increasing total propane concentration substantially over ambient levels. We consider only those propane tracer experiments ($n = 14$) in which the addition was >4 times the ambient level and consider the resulting rates from 24-hour incubations to represent the maximum propane-oxidizing potential for the basal population (fig. S5). Propane-oxidizing potentials were greater at locations with higher propane anomalies (Fig. 4A), sug-



gesting a priming effect wherein environmental exposure to propane induces increased propane-respiration capacity. In comparison, methane oxidation rates were generally low in the plume horizon, with a median value of just 10 nM d^{-1} ($n = 25$), 1 to 2 orders of magnitude too low to account for the oxygen anomalies. The plume at station H28 displayed an anomalously high methane oxidation rate of 820 nM d^{-1} , with cumulative methane consumption weakly supported by this location having the most ^{13}C -enriched methane ($\delta^{13}\text{C}$ - CH_4 of $-58.5 \pm 0.8\text{‰}$; $n = 3$) compared with all other locations sampled ($\delta^{13}\text{C}$ - CH_4 of $-61.3 \pm 2.2\text{‰}$; $n = 17$). A paucity of ethane and propane at

this location suggests extensive biodegradation, and comparison of methane oxidation rates to $\text{CH}_4/\text{C}_2\text{H}_6$ for all rate measurements reveals a positive exponential correlation (Fig. 4B). We interpret this relationship to reflect a slower substrate response and growth rate for methanotrophs relative to ethane degraders in the plume, although direct inhibition cannot be excluded. We suggest that the development of methanotrophic communities in deep hydrocarbon plumes is delayed with respect to that of ethane-, propane-, and butane-consuming communities.

To identify potential propane- and ethane-consuming bacteria active in the deep plumes, we collected cells and sequenced bacterial DNA from five locations containing distinctive propane and ethane anomalies. A cloning-based survey of the 16S rRNA gene was dominated by several sequences related to known hydrocarbon degraders—*Cycloclasticus* (21–24), *Colwellia* (25), and members of the *Oceanospirillaceae* (26)—indicating a low diversity bloom of hydrocarbon-oxidizing bacteria in the deep plumes. The plume closest to the wellhead had the highest levels of hydrocarbons and the least evidence for biodegradation and yielded the lowest proportion of putative hydrocarbon degraders (52%) relative to typical mesopelagic bacteria. We take this location to represent an early developmental stage in the bloom of hydrocarbon-oxidizing bacteria. The remaining four locations were each dominated by two clades of putative hydrocarbon degraders (Fig. 3B) related to *Cycloclasticus* and *Colwellia*, contrasting previous results (16) that found relatives of the *Oceanospirillaceae* as the dominant phylotypes. We suggest that the observed relatives of *Cycloclasticus* and/or *Colwellia* are blooming as a result of their capacity to consume propane, ethane, and potentially butane, although not at the exclusion of other bacteria or metabolisms. While *Cycloclasticus* is known for its ability to degrade aromatic compounds, sequences observed here are 90% similar to putative ethane and propane oxidizers identified by stable isotope probing (27), indicating the capability in this evolutionary lineage (Fig. 3C).

The extent to which various hydrocarbons may feed respiration and bacterial blooms depends on their concentration and bioavailability. Based on several assumptions (6), we calculate that methane, ethane, and propane released from the Deepwater Horizon leak will exert a biological oxygen demand in the deep plume horizon of up to 8.3×10^{11} g O_2 for methane respiration, 1.3×10^{11} g O_2 for ethane, and 1.0×10^{11} g O_2 for propane. In comparison, assuming that 968,000 barrels of oil were dispersed into the subsurface (5, 6), we calculate a maximum biological oxygen demand for oil of 4.4×10^{11} g O_2 . The sum of these values, $\sim 1.5 \times 10^{12}$ g of O_2 , provides an estimate of the maximum integrated deep-water O_2 anomaly expected from this event, with roughly 15% of the oxygen loss occurring in fresh plumes from respiration of propane and ethane. From these estimates, we predict that roughly two-thirds of the ultimate microbial productivity in deep plumes will arise

from metabolism of natural gas. We also predict boom-and-bust cycles of bacterial succession beginning with propane, ethane, and butane consumers, followed by the consumers of various higher hydrocarbons and methane. However, the plumes' bacterial population will also respond to persistent mixing of bacteria, oxygen, and hydrocarbons with nonplume waters, which could presumably lead to attenuation in the aging plumes.

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The Dynamics of the Onset of Frictional Slip

Oded Ben-David, Gil Cohen, Jay Fineberg*

The way in which a frictional interface fails is critical to our fundamental understanding of failure processes in fields ranging from engineering to the study of earthquakes. Frictional motion is initiated by rupture fronts that propagate within the thin interface that separates two sheared bodies. By measuring the shear and normal stresses along the interface, together with the subsequent rapid real-contact-area dynamics, we find that the ratio of shear stress to normal stress can locally far exceed the static-friction coefficient without precipitating slip. Moreover, different modes of rupture selected by the system correspond to distinct regimes of the local stress ratio. These results indicate the key role of nonuniformity to frictional stability and dynamics with implications for the prediction, selection, and arrest of different modes of earthquakes.

The relative motion of two contacting bodies under an imposed shear is governed by the ensemble of discrete contacts composing their interface (1). Although frictional slip (2, 3) is initiated by the rapid rupture of these contacts, understanding of the mechanisms of how interface rupture takes place is limited by our knowledge of the properties, strength, and stability of this rough interface.

Frontlike rupture modes bridge the gap between the microscopic interactions that define local frictional resistance and the resulting macroscopic motion imbued in the slip of large bodies

Rachael Institute of Physics, the Hebrew University of Jerusalem, Givat Ram, Jerusalem, Israel.

*To whom correspondence should be addressed. E-mail: jay@vms.huji.ac.il

(2–4). Laboratory experiments reveal three distinct modes of rupture: (i) slow ruptures, which propagate far below material wave speeds (5–8), (ii) “sub-Rayleigh” ruptures (5, 6, 9–12) that propagate up to the Rayleigh wave speed, and (iii) “supershear” rupture modes that surpass the shear wave speed C_S (5, 10, 12). Sub-Rayleigh ruptures are related to shear fracture, which is well understood theoretically (2); however, our understanding of the other rupture modes is much less clear. Supershear modes, which have long been considered theoretically possible in shear fracture (13–16), have only recently been observed (12), whereas our understanding of slow rupture modes is still very much in its infancy (17, 18). Each of these rupture modes may occur in earthquakes, but if, where, and how they are selected are still open questions. Although sub-Rayleigh modes are considered the most general mode of earthquake propagation (2, 4, 15), there is mounting evidence for the importance of both slow (19–21) and supershear (22, 23) rupture modes along natural faults.

To understand how properties of frictional rupture couple to the profiles of local stresses along a frictional interface, we conducted experiments with acrylic poly(methyl-methacrylate) blocks in two qualitatively different loading systems (fig. S1) (24). At the start of each experiment, the blocks were pressed together by a normal force F_N (Fig. 1A). External shear forces, F_S , were applied to either the trailing edge ($x = 0$) of

the top-block, uniformly along the bottom block, or in a mixture of the two. F_S was incremented quasi-statically, eventually triggering stick-slip sliding. We continuously performed simultaneous measurements of the real contact area, $A(x, t)$, at every point x at rates of up to 250,000 samples/s (here, t is time). In parallel, shear and normal stress profiles adjacent to the interface, $\tau(x)$ and $\sigma(x)$, respectively, were measured every second. All rupture events encompassing either partial sections of the interface (6) or the entire interface were measured.

In nearly all frictional systems, $\tau(x)$ and $\sigma(x)$ are highly nonuniform, suggesting that nonuniformity is generic. Even in ideal laboratory systems, large stress nonuniformity can be produced by minute interface curvature, differences in the materials and/or geometries of the contacting bodies, or dynamically, by earlier slip events (6, 17). In the example presented in Fig. 1, B and C, we show how geometrically dissimilar blocks generate large shear stress variations at an optically flat interface under uniform application of F_N (Fig. 1). Because the dimensions of the upper and lower blocks are not identical, differential Poisson expansion is countered by pinning of the blocks at the frictional interface (25). This generates shear stresses even for $F_S = 0$. Additional large stress variations result from any torque when $F_S > 0$ is applied.

Controlled variations of loading conditions (24) lead to spatial variations of $\tau(x)$ and $\sigma(x)$ that produced the full range of rupture modes (Fig. 2).

These include individual events (Fig. 2, A to C) where slow, sub-Rayleigh, and supershear ruptures or combinations of these modes (Fig. 2A) took place. Additionally, the dynamics of rupture can change in successive events within a single stick-slip sequence (Fig. 2, D to F). All events in this sequence nucleated at approximately the same location ($x \sim 150$ mm) and propagated in both directions. In the first stick-slip event (Fig. 2D), the left-traveling front initiated at near-shear velocity, then continually slowed until ultimately arresting at $x \sim 50$ mm. In the second event, the left-traveling front initiated beyond the shear-wave speed (~ 1600 m/s), slowed to sub-Rayleigh propagation (250 to 500 m/s) near the sample edge, but did not arrest. The left-propagating front in the third event initiated at a super-shear velocity (~ 2300 m/s) and traversed the entire interface without slowing down. The rupture direction need not correspond to the resulting slip, which is determined by the loading.

Comparison of the rupture velocities to the local stress ratio, $\tau(x)/\sigma(x)$, suggests that this quantity is strongly coupled to the local front dynamics (Fig. 2). The local propagation speeds consistently increase with $\tau(x)/\sigma(x)$, with front arrest occurring when $\tau(x)/\sigma(x)$ falls below ~ 0.5 (for example, Fig. 2A at $x = 50$ mm). This qualitative dependence is both local along the interface and independent of how the local stress ratios were imposed. Supershear fronts can also nucleate under quasi-static external loading (Fig. 2, C and F). These ruptures can abruptly initiate (Fig. 2C), even when preceded by a slow, gradual nucleation process.

The local propagation velocities $V(x)$ of 287 different fronts as a function of $\tau(x)/\sigma(x)$, measured before slip initiation, demonstrate the generality of these observations (Fig. 3). Each front is part of a system-sized event, with each $V(x)$ indicating the instantaneous rupture velocity as a front traverses a specific strain gage. If we consider only fronts traversing locations far from the system's loading points or free edges, the data collapse onto a rough curve whose form indicates three distinct regimes of rupture dynamics: (i) slow fronts [$\tau(x)/\sigma(x) < 0.5$], (ii) sub-Rayleigh fronts [$0.5 < \tau(x)/\sigma(x) < 0.8$], and (iii) supershear rupture [$\tau(x)/\sigma(x) > 0.8$]. The data collapse occurred for widely different external loading conditions (24), including simple edge-loading, a combination of uniform shear and edge loading, and uniform shear loading (compare with Figs. 1 and 2). This suggests that rupture-mode selection is coupled to $\tau(x)/\sigma(x)$, though it is not explicitly dependent on how loads are applied. The data fail to collapse in regions (for example, near loading points) where stress gradients are so large that stresses at the interface do not mirror those relieved in the local vicinity of the rupture tip.

At loading levels that are even incrementally below that needed to precipitate each slip event in Fig. 3, no slip occurred. Thus, the system was stable for values of $\tau(x)/\sigma(x)$ that far exceeded the static-friction coefficient (26) $\mu_S = F_S/F_N \sim 0.5$. This is surprising, as it is generally believed (3) that the value of μ_S is the criterion for stability to

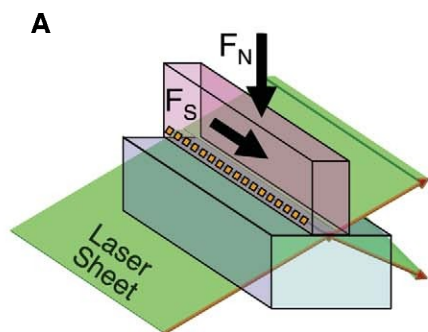
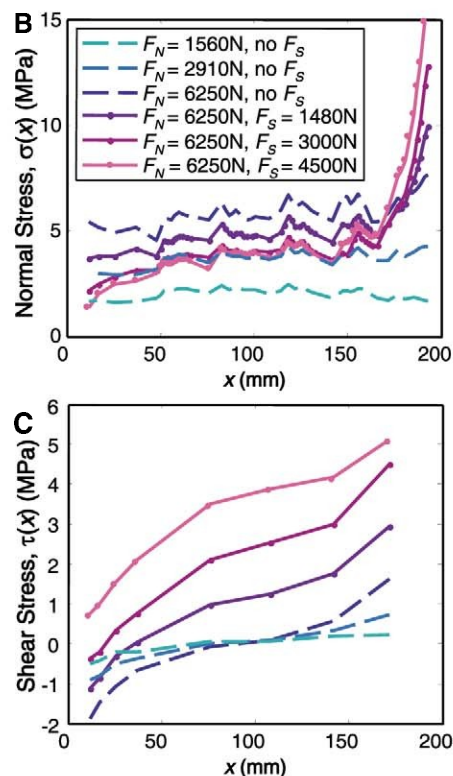


Fig. 1. Inhomogeneous normal and shear stresses are ubiquitous. (A) The real contact area $A(x, t)$ is measured by light transmission along the interface, whereas shear and normal stresses are measured adjacent to the interface (orange squares). Different loading configurations are used [details in (24)]. An example of the evolution of $\sigma(x)$ (drawing not to scale) (B) and $\tau(x)$ (C) profiles for uniformly applied shear and normal forces. This loading configuration led to the rupture event depicted in Fig. 2C. Stress profiles (dashed lines) measured during the application of F_N with $F_S = 0$; subsequent profiles (solid lines) were measured when F_S was applied for a fixed $F_N = 6250$ N. Measurement points are connected by lines for clarity. Application of a uniform normal stress with $F_S = 0$ creates a nonuniform antisymmetric $\tau(x)$ profile, solely as a result of differential Poisson expansion frustrated at the interface. Application of F_S increases the mean level of $\tau(x)$ while producing strong nonuniformity of $\sigma(x)$ near the block edges to compensate for external torque.



frictional motion at any point along a frictional interface. Our experiments demonstrate that this assumption is not valid; interfaces are locally stable even for local values of $\tau(x)/\sigma(x)$ that exceed $4\mu_s$ (Fig. 3). Although $\tau(x)/\sigma(x)$ can locally exceed μ_s , the integrated values of $\tau(x)$ and $\sigma(x)$ are consistent with the value of μ_s in each experiment.

When considering a frictional interface, it is tempting to simplify the problem by considering uniform stress profiles along the interface (I). Any geometrical or material mismatch, however, as well as the existence of edges, will lead to substantial nonuniformity (see, for example, Fig. 1). One can approximate uniform stress profiles only under a limited set of carefully controlled conditions (12, 26). Thus, nonuniform stress profiles are the rule and are present on nearly any naturally occurring (4) or engineered frictional system. Along natural faults, heterogeneous profiles of $\sigma(x)$ or $\tau(x)$ can have a variety of additional origins. These include material heterogeneity of either shear strength or elastic moduli of the host material, inelastic deformations near fault tips, or spatial gradients of applied stress fields (3). Stress inhomogeneity can further evolve dynamically, through the partial release and transfer of stresses between slip patches (2, 4, 15) or by finite slip events driven by inhomogeneous stress application (6, 17).

We can intuitively understand why the local stress ratio is tightly coupled to the rupture mode (15). We know that a crack propagates when the strain energy released in the bulk medium exceeds its fracture energy (27): the energy needed to create a new unit surface. At a frictional interface, this effective energy cost is proportional to the real area

of contact $A(x)$, which is, in turn, locally proportional (I) to $\sigma(x)$ at each point x . Here, the effective fracture energy Γ is not a material-dependent quantity as in the fracture of bulk materials, but instead reflects the local strength of the interface, as determined by $\sigma(x)$. On the other hand, $\tau(x)$ is a locally measured quantity that mirrors the density of strain energy stored, before the arrival of a rupture front, within a region of finite size surrounding the point, x . Thus, at locations sufficiently removed from regions with high stress gradients, $\tau(x)/\sigma(x)$ reflects the balance between the potential energy available, before rupture, in the vicinity of each point and the energy needed to rupture the interface.

Fig. 3. Rupture-mode selection depends on $\tau(x)/\sigma(x)$. Local propagation velocities $V(x)$ as a function of $\tau(x)/\sigma(x)$ for 287 different fronts in system-sized slip events for edge-loading (diamonds) and (predominantly) uniformly applied shear (circles); see inset. The rough data collapse indicates three regimes in which $\tau(x)/\sigma(x)$ correlates well with the slow, sub-Rayleigh, and supershear rupture modes. Note that the local stress ratio $\tau(x)/\sigma(x)$ may far exceed the macroscopic static-friction coefficient $\mu_s \sim 0.5$. To avoid the effects of large stress gradients, all measurements were performed at strain-gage rosettes located away from sample edges at $x = 108$ mm (red), 142 mm (green), 172 mm (blue), 77 mm (magenta), 108 mm (yellow), and 142 mm (light blue). $V(x)$ was obtained from the contact area measurements surrounding these locations. Dashed lines indicate longitudinal (C_L) and shear (C_S) wave speeds.

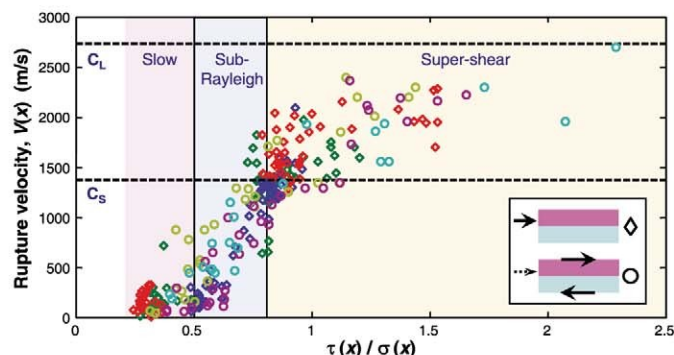
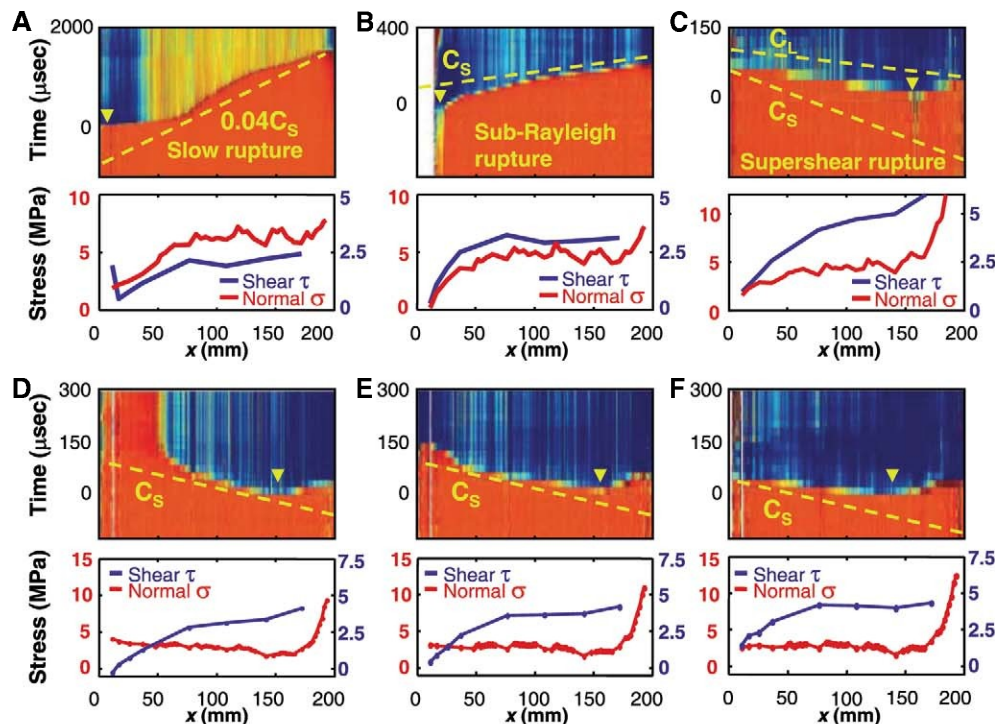


Fig. 2. Local stress profiles dramatically influence rupture dynamics. (Top panels) Changes in $A(x,t)$ normalized by $A(x,t = -1 \text{ msec})$. Hotter (or colder) colors denote increased (or reduced, respectively) contact area. Rupture fronts are identified by a sharp change in color. Dashed lines denote sound speeds: $C_S = 1370$ m/s (shear) and $C_L = 2730$ m/s (longitudinal). (Bottom panels) Corresponding stress profiles before each event. Normal stress, $\sigma(x)$ (red lines), and shear stress, $\tau(x)$ (blue lines), are shown. Nucleation occurred at $t = 0$ at locations denoted by yellow arrowheads. (A to C) The three different rupture modes: slow (A), sub-Rayleigh (B), and super-shear (C). Events were generated in system II (24) where (A) the optional stopper was used, (B) the stopper was not used, or (C) the loading described in Fig. 1B was used. In (A) and (B), frustrated Poisson expansion was minimized by nonuniform application of $\sigma(x)$. (C) Note how the slow nucleation phase at $x \sim 150$ mm rapidly transitions to super-shear rupture. (D to F) Three successive slip events within the same stick-slip sequence as F_5 was quasi-statically increased (loading conditions similar to Fig. 1). Events range from front arrest (D) to supershear rupture (F). Note that local stress ratios dramatically affect rupture dynamics: front arrest for $\tau(x)/\sigma(x) < 0.5$, slow rupture for $\tau(x)/\sigma(x) \sim 0.5$, sub-Rayleigh propagation for $\tau(x)/\sigma(x) \geq 0.5$, and super-shear rupture propagation where $\tau(x)/\sigma(x)$ is significantly larger than 0.5.



tially nonuniform systems when highly stressed regions are encountered; however, we do not yet fully understand what causes rupture nucleation in our system. We find that nucleation locations are often regions where $\tau(x)/\sigma(x)$ is maximal (for instance, see Fig. 2C). Hence, either low $\sigma(x)$ or high $\tau(x)$ can influence the location and initiation of rupture fronts. Increased $\sigma(x)$, as at the leading edge in Fig. 2F, will serve to suppress nucleation. Likewise, values of $\tau(x)$ that oppose the applied shear, such as at the trailing edges of Fig. 2, will have the same effect. The high values of both $\sigma(x)$ and $\tau(x)$ associated with corners can either make edges susceptible to rupture nucleation (Fig. 2A) or suppress rupture, depending on the competition between them.

Once a rupture front is nucleated, knowledge of the local stress profiles along the interface allows us to predict the rupture mode and could indicate when a rapid mode will either arrest to a complete stop (Fig. 2A) or evolve into a slow front (Fig. 2B). Thus, the initiation/transition locations of the slow fronts observed in previous studies (5, 6) become clearer. Such questions of predictability are important when applied to understanding earthquake dynamics (3). Although $\tau(x)/\sigma(x)$ is an elusive quantity to measure along natural faults, indirect measurements may be possible by coupling precise measurements of spatial variations of $V(x)$ to laboratory measurements of the $\tau(x)/\sigma(x)$ dependence on V , as in Fig. 3. This

estimate of this otherwise inaccessible quantity could provide some measure of predictability of the eventual size and dynamics of fast earthquakes along natural faults.

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Block Copolymer Self-Assembly–Directed Single-Crystal Homo- and Heteroepitaxial Nanostructures

Hitesh Arora,^{1,2*} Phong Du,^{1†} Kwan W. Tan,¹ Jerome K. Hyun,^{3‡} John Grazul,⁴ Huolin L. Xin,³ David A. Muller,^{5,6} Michael O. Thompson,¹ Ulrich Wiesner^{1§}

Epitaxy is a widely used method to grow high-quality crystals. One of the key challenges in the field of inorganic solids is the development of epitaxial single-crystal nanostructures. We describe their formation from block copolymer self-assembly–directed nanoporous templates on single-crystal Si backfilled with Si or NiSi through a laser-induced transient melt process. Depending on thickness, template removal leaves either an array of nanopillars or porous nanostructures behind. For stoichiometric NiSi deposition, the template pores provide confinement, enabling heteroepitaxial growth. Irradiation through a mask provides access to hierarchically structured materials. These results on etchable and non-etchable materials suggest a general strategy for growing epitaxial single-crystal nanostructured thin films for fundamental studies and a wide variety of applications, including energy conversion and storage.

Most nanostructured inorganic materials directed by organic molecule self-assembly are either amorphous or polycrystalline (1–7). One of the key remaining issues in the field is the development of single-crystal nanostructured inorganic materials with an epitaxial relation to an underlying substrate. Such materials may enable advances in areas such as energy generation and storage. For example, the charge carrier

mobility in crystalline materials in small dimensions is substantially reduced by grain boundaries, which decreases charge collection efficiency in photovoltaic cells (8). Although large-area patterned inorganic epitaxial single crystals have been synthesized before, the patterns have been limited to the micrometer scale (9). Several attempts have been made to achieve smaller-scale structures. Block copolymer thin films have been used as etching masks to

generate inorganic nanostructures, but the process depends on etchable substrates, and the features can only be vertically aligned and are always open from the top (10–13). Laser-induced melting of Si and subsequent infiltration into a quartz mold have been previously described (14). The process relies on so-called top-down lithography and etching to make a mold, however, with a structural period of hundreds of nanometers. Moreover, the epitaxial relation of the resulting structures to the substrate was not elucidated, and the heteroepitaxy of non-etchable materials was not addressed. Directional rapid solidification and epitaxy have been described for semicrystalline block copolymer films, but without any inorganic materials (15). Hierarchically ordered oxide structures have been demonstrated before, but the oxides were not single-crystalline (16). Mesoporous, single-crystalline,

¹Department of Materials Science and Engineering, Cornell University, Ithaca, NY 14853, USA. ²School of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY 14853, USA. ³Department of Physics, Cornell University, Ithaca, NY 14853, USA. ⁴Cornell Center for Materials Research, Cornell University, Ithaca, NY 14853, USA. ⁵School of Applied and Engineering Physics, Cornell University, Ithaca, NY 14853, USA. ⁶Kavli Institute at Cornell for Nanoscale Science, Cornell University, Ithaca, NY 14853, USA.

*Present address: Intel Corporation, Chandler, AZ 85226, USA. †Present address: DuPont Chemical Company, Wilmington, DE 19880, USA.

‡Present address: Department of Materials Science and Engineering, Northwestern University, Evanston, IL 60208, USA. §To whom correspondence should be addressed. E-mail: ubw1@cornell.edu

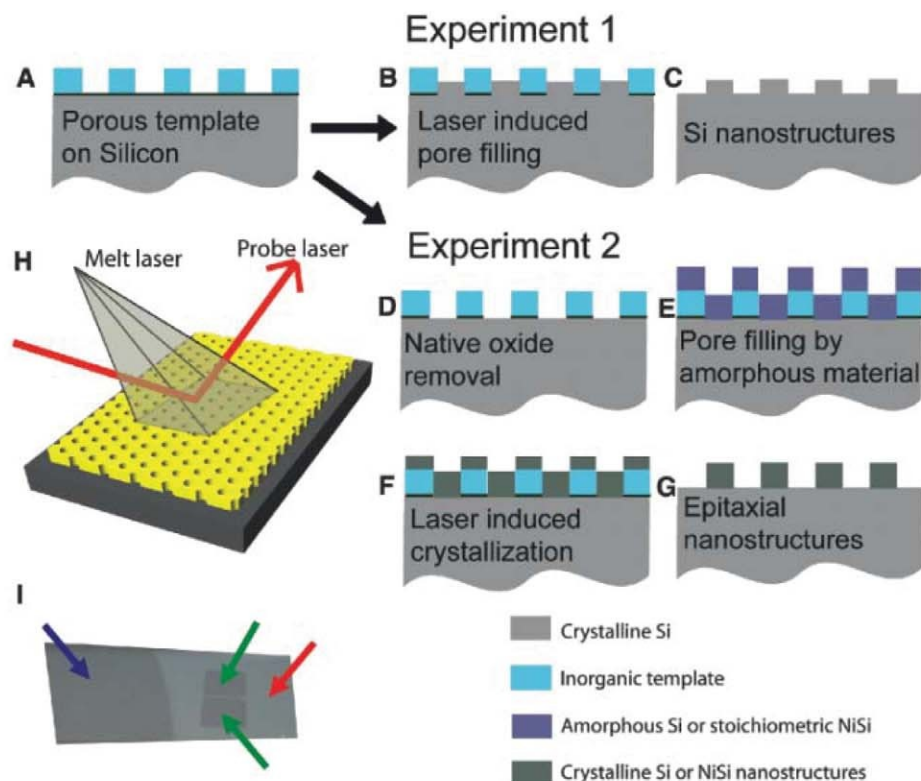


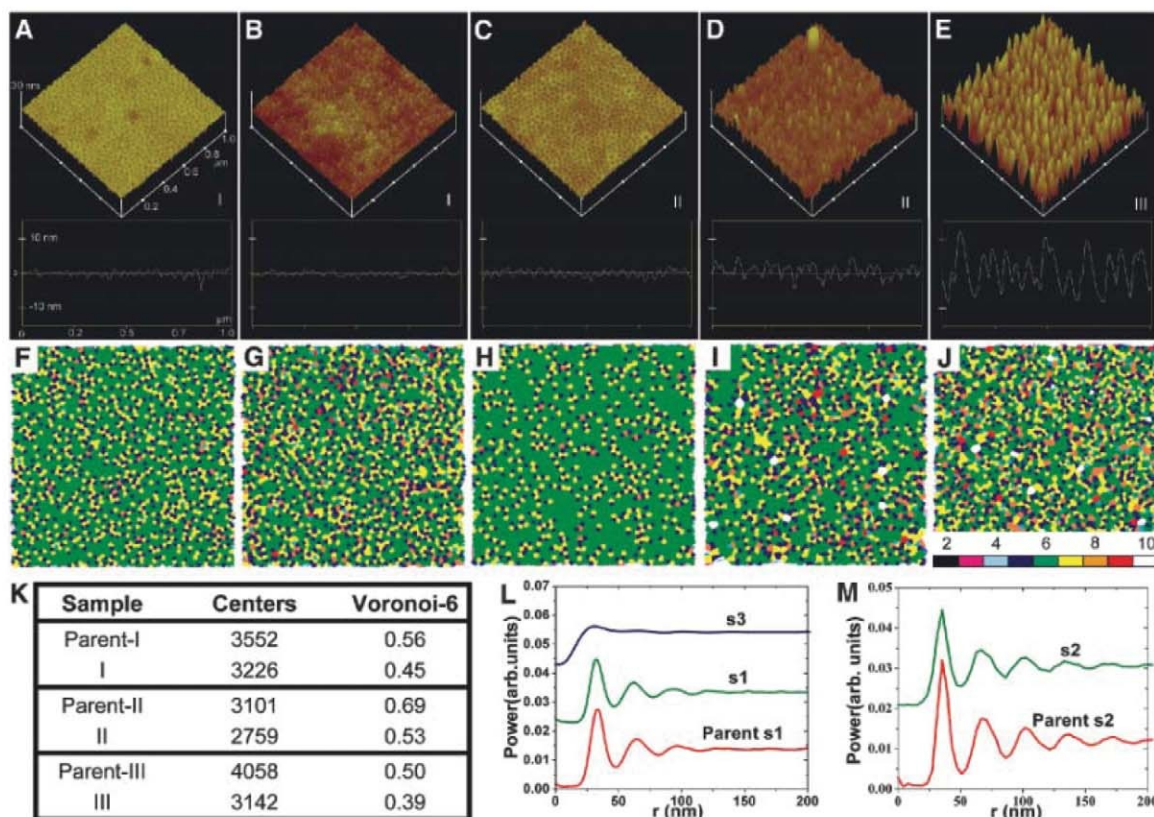
Fig. 1. Schematic of single-crystal homoepitaxial Si and heteroepitaxial NiSi nanostructure generation. (A) Porous template. (B and C) First experimental process. (D to G) Second experimental process. (H) Laser irradiation schematic. (I) Photograph of Si wafer with bare crystalline Si substrate (blue arrow), with a-Si deposited (red arrow) and spots after laser-induced crystallization (green arrows).

mixed transition-metal-oxide particles and calcite single crystals with gyroid morphology have been generated from surfactant and polymer self-assembly, respectively, but their epitaxial relations to substrates have not been described (17–19). Finally, epitaxial thin-film oxide growth on single-crystal substrates and subsequent topotactic crystal transformation with substantial volume shrinkage leading to pores with crystallographic alignment have recently been demonstrated. The process did not involve organic molecule self-assembly, however, and the topotactic transformation process is most likely materials-specific and thus may not easily be generalized to other materials (20).

In this work, we used nanoporous thin films ~15 to 100 nm thick on Si to define and control epitaxial crystallization of Si and NiSi, with templates having periodicities on the scale of tens of nanometers. Template thin films were obtained in a bottom-up self-assembly approach using inorganic precursors, a process directed by block copolymer self-assembly. Template pores were filled with amorphous Si or NiSi (a-Si or a-NiSi). Subsequently, laser-induced melting converted the amorphous phase into crystalline materials. Depending on template thickness, either arrays of isolated nanopillars or interconnected three-dimensional nanostructures were generated.

The processes we used are schematically shown in Fig. 1, A to H. In the first part of this study, bare Si wafers were spin-coated with a mixture of prehydrolyzed metal alkoxides [(3-glycidyloxypropyl-)trimethoxysilane and aluminum sec-butoxide, in

Fig. 2. AFM images with respective height profiles. (A and C) Porous templates. (B, D), and (E) Si nanostructures. (F to J) Voronoi analysis of corresponding AFM images. (K) Table for samples s1 (I), s2 (II), and s3 (III). RDFs of (L) samples s1 and s3 and (M) sample s2 are shown. The scan size for all AFM images is $1 \times 1 \mu\text{m}$, and the scale bar (y axis) ranges from –15 to 15 nm in the height profiles. The scan area for Voronoi analysis is $2 \times 2 \mu\text{m}^2$.



a ratio of 8:2 by weight] and poly(isoprene-*b*-ethylene oxide) (PI-*b*-PEO) diblock copolymers (ratio of metal alkoxides to polymer, 6:1 by weight) in organic solvents (tetrahydrofuran:chloroform, 1:1 by weight) (21). Monolayers with inverse hexagonal nanostructure were formed (22), with one inorganic-rich domain (PEO + inorganic) and the other purely organic (PI). The organic components were subsequently removed by slow heating to 500°C, leaving an ordered nanoporous hexagonal array, with pores accessible from the top, Fig. 1A. For the block copolymers we used, the nanopore lattice spacings were between 30 and 35 nm, depending on the polymer molecular weight. The physical film thickness after calcination was 1.6 ± 1.6 nm, as determined by a combination of scanning electron microscopy (SEM) and x-ray diffraction (22). The nanopores were initially filled with Si through the use of 40-ns XeCl excimer pulsed laser irradiation [wavelength (λ) = 308 nm] (Fig. 1H). The template itself was transparent, with the laser light being absorbed by the Si substrate. Time-resolved reflectance (TRR) of the sample surface was monitored with a diode probe laser (λ = 650 nm)

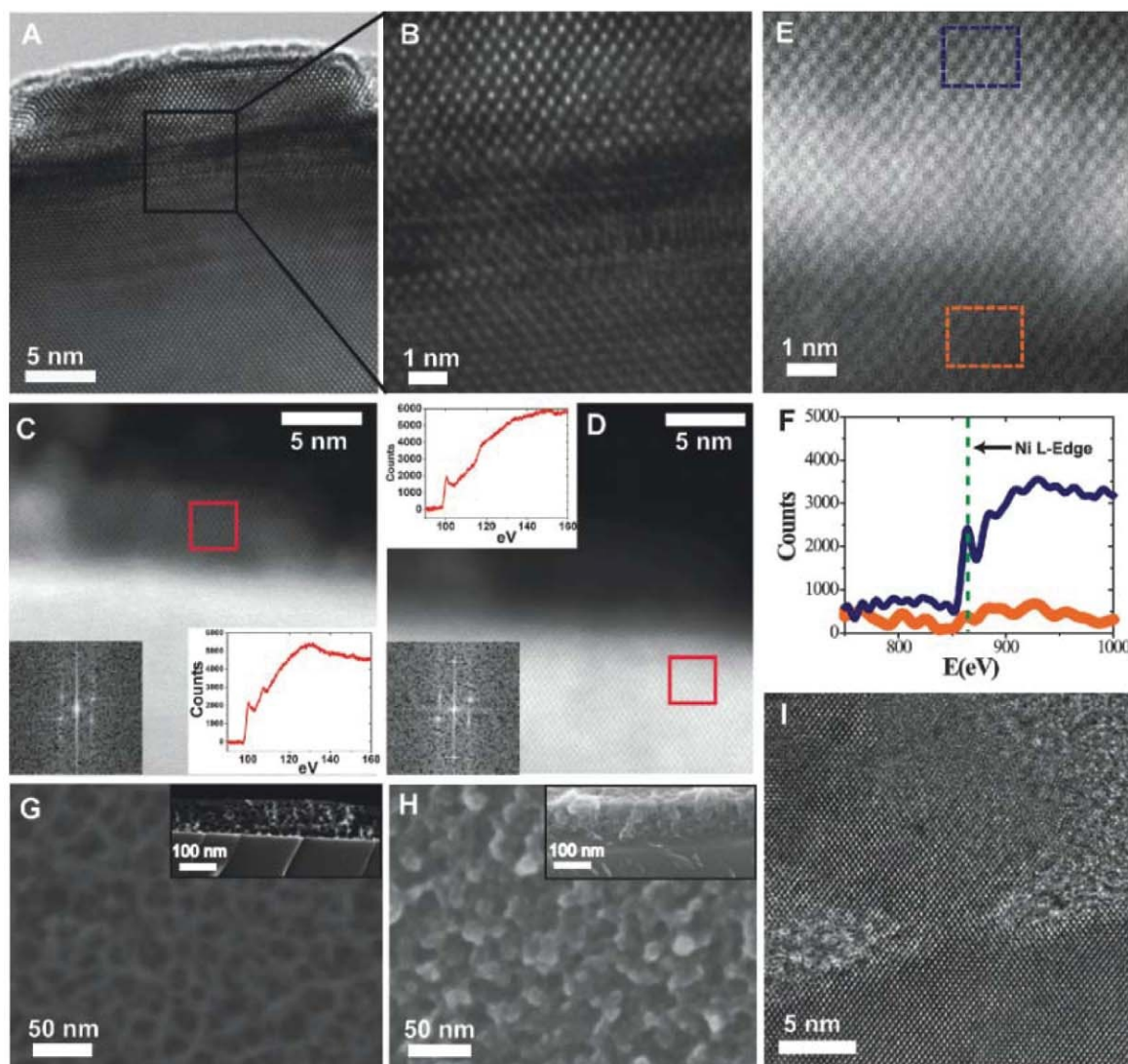
and was used to measure melt duration. At fluences (σ) above the melt threshold (~ 600 mJ/cm² for Si), the semiconducting solid became a metallic liquid, and the surface reflectance increased sharply (23), providing a fingerprint for understanding the mechanistic details of the process.

In first experiments, with sample s1, the porous template was irradiated with the excimer laser at fluences sufficient to melt the underlying Si substrate. Because of rapid cooling into the substrate, the total melt duration was only ~ 20 to 100 ns, and the total time at higher temperatures was less than a few microseconds, preventing sintering collapse of the porous silica-type template. After laser irradiation, the aluminosilicate template was dissolved away with a 48% hydrofluoric acid (HF) solution to expose the resulting array of Si nanopillars (Fig. 1, B and C). Figure 2, A and B, show atomic force microscopy (AFM) images of sample s1 before and after irradiation with five laser pulses of 40 ns duration and the subsequent removal of the skeletal aluminosilicate. The AFM results suggest excellent pattern transfer from the template into Si. The images were quantitatively

analyzed with analysis tools described elsewhere (24, 25). Figure 2, F and G, show representative Voronoi diagrams of the aluminosilicate template and the resulting nanostructured Si array, respectively. The Voronoi diagram represents the number of nearest neighbors of a pore or resulting Si nanostructure after melting and is color-coded to facilitate identification of defects and grain boundaries (25). The Voronoi-6 ratio, defined as the fraction of pores or resulting Si nanostructures with sixfold hexagonal nearest-neighbor symmetry, provides a simple metric for sample comparison. As indicated by the increased number of defects in the Voronoi diagram, the transformation from pores to Si nanopillars increases disorder in the system. However, transfer of the template into Si structures was accomplished with a high yield, with greater than 90% conversion of pores to Si nanopillars and only a 20% decrease in the Voronoi-6 ratio (Fig. 2K, sample s1).

Although in these experiments the transfer fidelity was high in the lateral dimensions, section analysis of AFM images showed the resulting Si nanostructures to be only 1 to 2 nm

Fig. 3. EM images of homo- and heteroepitaxial nanostructures. (A and B) TEM images of Si nanostructure cross sections. The phase contrast reversals at the interface result from thickness changes in the sample: The substrate is thicker than the pillar, and the electrons undergo a larger phase shift in the substrate. STEM images, which do not suffer from contrast reversals with thickness, are shown for (C) nanostructure in focus and (D) Si substrate in focus. Corresponding EELS spectra and FFT insets are in (C) and (D). (E) STEM image of a NiSi pillar cross section on Si. (F) EELS spectra on the NiSi pillar and Si substrate from boxed regions in (E). (G) Top view and cross-sectional (inset) SEM images of the ~ 100 -nm-thick porous niobia template. (H) Top view and cross-sectional (inset) SEM images of the porous Si nanostructure obtained from (G). (I) Cross-sectional TEM image of the thicker porous Si nanostructure in (H).



high (see height profile in Fig. 2B). This motivated the development of a second, modified, process in subsequent experiments, enabling better filling of the pores and thus forming taller Si nanopillars. To this end, an a-Si overlayer (Fig. 1, D to G) was deposited onto the template before laser irradiation. Furthermore, the native oxide present at the bottom of the pore was first removed by Ar ion sputtering before a-Si deposition in the same chamber and at the same base pressure. This resulted in a clean interface between deposited a-Si in the pore and the crystalline Si (c-Si) substrate (Fig. 1E). Laser irradiation at an energy density above the melt threshold of c-Si of sample films (sample s2) prepared in this way resulted in epitaxial crystallization of Si nanopillars from the substrate inside the pore (Fig. 1F). Subsequent HF (48%) treatment removed the oxide template, leaving behind an array of single-crystal nanopillars (Fig. 1G). The AFM image and Voronoi analysis (Fig. 2, C and D, and H and I, respectively) of the template and pillar array confirmed 89% pattern transfer and only a 23% decrease in the Voronoi-6 ratio (Fig. 2K, sample s2). The average height of pillars was found to be 6 to 7 nm, which is substantially higher than before (see height profile in Fig. 2D).

The epitaxial crystallinity of the Si nanopillars was confirmed by imaging pillar cross sections with high-resolution TEM and scanning TEM (STEM) (Fig. 3). The presence of lattice fringes in the pillars in congruence with the fringes in the substrate (Fig. 3, A and B) shows the crystalline nature of the nanopillars. The epitaxial growth of the nanopillars was confirmed by STEM-mode imaging, in which the Si substrate and nanopillar are both shown to be aligned along the [110]

zone axis (Fig. 3, C and D). The two-dimensional fast Fourier transform (FFT) patterns on the pillar and substrate (lower left insets in Fig. 3, C and D, respectively) show that the two areas share the [111] reflections. Because of an offset in depth that exceeded the depth of field of the microscope for 0.3-nm fringes, STEM-mode images were taken with only the pillar (Fig. 3C) and only the substrate (Fig. 3D) in focus. Electron energy-loss spectroscopy (EELS) on the pillars (lower right inset in Fig. 3C) revealed the presence of a crystalline Si *L*-edge peak at 100 eV, also seen in the spectrum of the c-Si substrate (upper left inset in Fig. 3D). The small peak at 108 eV (inset of Fig. 3C) arises from Si in the thin SiO₂ surface layer that forms in air after HF treatment.

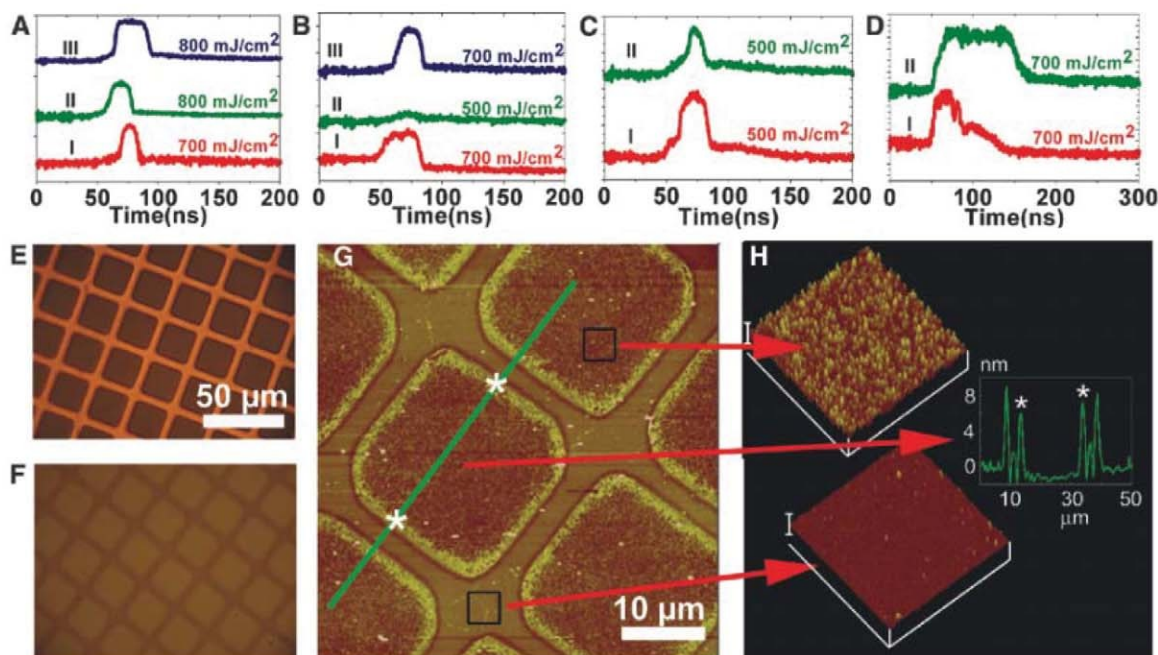
Because nanopores generated through block copolymer self-assembly laterally confine the crystal growth to narrow dimensions, it is possible to heteroepitaxially grow crystals with moderate lattice mismatch on Si. In this way, single-crystal nanostructures can be generated that are not easily accessible through etching. To this end, stoichiometric NiSi was sputter-deposited on the porous template (deposition rates were monitored with a crystal monitor) after the native oxide was removed. The NiSi (202) lattice mismatch to Si (220) was 0.5 to 0.6%, and in the absence of lateral confinement, NiSi film deposition on Si leads to multiple lattice orientations (26) and small-island formation, releasing the strain accumulated over larger lateral film distances. The nanoporous NiSi-filled films were then laser-irradiated to melt the amorphous NiSi, which subsequently solidified epitaxially from the substrate (27). The short duration of the melt minimized Ni diffusion or the incorporation of Si from the substrate into the liquid

phase, thus maintaining the correct stoichiometry in the pore. The epitaxial orientation of the strained NiSi lattice on the c-Si substrate was confirmed by STEM images of the NiSi/Si interface, with the substrate aligned along the [110] zone axis (Fig. 3E). The EELS spectra collected on the pillar and the substrate clearly indicated the presence of a Ni *L*-edge peak on the pillar and an absence of Ni below the interface (Fig. 3F). The pore sizes generated through block copolymer self-assembly thus provided the necessary confinement for heteroepitaxy growth on single-crystal substrates for materials with moderate lattice mismatch.

We next examined the mechanistic aspects of the single-crystal nanopillar formation. A series of experiments was first performed (sample s3) in which the a-Si was deposited without etching the native oxide layer, thereby preventing direct growth from a c-Si substrate. The native oxide layer is expected to prevent molten Si from flowing through the pores, and hence deposited Si should remain confined to the pores (or on top of the template) when melted by the excimer laser pulse. This in turn should result in Si nanopillars of maximum height (~15 nm). This was indeed observed (see the AFM analysis in Fig. 2E). Voronoi analysis of AFM data revealed a 77% pore-to-pillar conversion, with a 22% decrease in the Voronoi-6 ratio (Fig. 2, J and K, sample s3).

For further quantitative data analysis, the radial distribution functions (RDFs) of the AFM images were computed for the parent templates and the resulting Si pillar nanostructures (Fig. 2, L and M). The RDFs of the unirradiated, parent templates exhibited narrow first-order maxima and multiple higher-order peaks. With no deposited films (sample s1), after irradiation the RDF

Fig. 4. Elucidation of the formation mechanism of single-crystal nanostructures. (A) TRR signal on (I) bare Si at 700 mJ/cm², (II) bare Si at 800 mJ/cm², and (III) sample s1 at 800 mJ/cm². (B) a-Si deposited on bare Si (I), first irradiation at 700 mJ/cm²; (II) second irradiation at 500 mJ/cm² after (I); (III) second irradiation at 700 mJ/cm² after (I). (C) a-Si deposited on bare Si (I), first irradiation at 500 mJ/cm²; (II) second irradiation at 500 mJ/cm² after (I). (D) Sample s2 (I), first irradiation at 700 mJ/cm²; (II) second irradiation at 700 mJ/cm² after (I). Optical micrograph (E) of a copper TEM grid and (F) the resultant pattern on sample s3 (with deposited amorphous Si over the template) after laser exposure. (G and H) AFM images after HF treatment. Scan size is 2 × 2 μm, and the I bar along the z axis is 40 nm in (H).



exhibited very similar characteristics, with only slightly less power in each peak because of the slightly reduced ordering. In contrast, samples with deposited films where native oxide was not removed behaved quite differently (sample s3). The RDF of the resulting Si nanopillars exhibited a first-order maximum shifted to larger distances [pillar-to-pillar distance (r) = 39 nm] as compared to the template (r = 31 nm) and a rapid decay with no additional higher-order peaks. Examination of the AFM image in detail revealed sequences of pillars with the expected nearest-neighbor distance (r = 31 nm), surrounded by neighbors with distances closer to the expected second-nearest-neighbor value. This suggests that a substantial number of pores simply did not get filled, which is consistent with the 37% loss of centers from the Voronoi analysis. The corresponding RDF maximum indeed lies in between the nearest- and second-nearest-neighbor spacings and may be caused by a slight deformation of the template toward the empty space during melting.

The Si transient melt behavior during laser irradiation was monitored with TRR of samples. When irradiated above the melt threshold, the reflectance of a bare c-Si substrate will jump as the melt first forms, remain constant while the melt front propagates into the substrate, and decrease abruptly only as the surface solidifies (Fig. 4A, I). Increasing the fluence from 700 to 800 mJ/cm² extends the melt duration (that is, there is a broader reflectance curve) (Fig. 4A, II). Small changes in the reflectance before melting result from simple surface heating (28). We investigated three sample structures: (i) the c-Si substrate with the porous template, (ii) the c-Si substrate with a-Si on top, and (iii) the c-Si substrate with the template and a-Si deposited on top. With just the porous template (case i), the TRR signal remained very similar to that of the bare c-Si substrate, although the melt duration increased by ~8 ns at 800 mJ/cm² (compare traces in Fig. 4A, II and III). On the time scale of the laser pulse and melt duration, the cross-linked silica-type template does not undergo any substantial structural change. The extended melt duration arises partially from the antireflective nature of the porous template coating (29), but also suggests the incorporation of a low concentration of impurities (possibly O or Al) during solidification.

In case ii, with a-Si deposited directly on the c-Si substrate, the TRR signal strongly depended on the fluence (Fig. 4, B and C). With a lower melting temperature, the melt threshold for a-Si is less than that of c-Si (30). Epitaxial solidification can occur only for fluences sufficient to melt into the c-Si substrate (Fig. 4B), with polycrystalline Si (poly-Si) resulting at lower fluences (Fig. 4C) (31). Transformation of the a-Si could be readily confirmed visually (Fig. II). After irradiation at or above 700 mJ/cm², samples behaved similar to the bare substrate, with a melt threshold near 600 mJ/cm² (Fig. 4B, I to III). However, for initial irradiations below the melt threshold for c-Si (e.g., at 500 mJ/cm²), a peak was observed in the TRR signal (Fig. 4C, I). When the spot was ir-

radiated again at this fluence, the peak remained (Fig. 4C, II), indicating the formation of poly-Si structure with lower thermal conductivity.

From these results we concluded that in order to epitaxially crystallize a-Si deposited in the template pores (case iii), samples had to be irradiated above the melt threshold of c-Si. Upon irradiation (Fig. 4D, I), the reflectance first increased rapidly, then stayed constant, followed by a steep first drop; and finally further decreased gradually over several tens of nanoseconds. The drop in reflectance in two steps is probably due to the different time scales involved for heat transfer from molten Si to the substrate within a pore (faster) and at the top of the template, which is a poor heat conductor (slower). During a second irradiation at the same spot (Fig. 4D, II), the reflectance signal exhibited a single peak and decay, suggesting the absence of Si on top of the template. Because in various experiments more a-Si was deposited than was necessary to fill the pores, we speculate that this could only happen if the deposited Si on top of the template flowed through the pores to move under the template, displacing the template upward. The poor wetting properties (32) between molten Si and the aluminosilicate template might propel this surface tension-driven flow of molten Si through the template.

A key step in obtaining single-crystal epitaxy is to remove the native oxide layer to generate a clean interface between deposited amorphous material in a pore and the c-Si substrate. Maintaining this condition should enable the generation of single-crystal epitaxial nanostructures from thicker self-assembled porous templates. Argon ion sputtering removes oxide layers in direct line of sight from the top only. One challenge in working with silica-type structures is the need to remove the native oxide to enable the epitaxial crystallization, while not simultaneously removing the template. In order to work with thicker and more complex-shaped structures, we moved to a porous amorphous niobia template coupled with plasma-enhanced chemical vapor deposition (PECVD) to fill the porous structures with amorphous inorganic material. Figure 3G shows SEM images of a roughly 100-nm-thick porous amorphous niobia template with network structure. These templates were generated in a similar way as the aluminosilicate templates, using a poly(isoprene-*b*-styrene-*b*-ethylene oxide) (PI-*b*-PS-*b*-PEO, ISO) triblock copolymer with a molecular mass of 23,180 g/mol [17.2 volume % (vol %) PEO, 52 vol % PS; polydispersity index = 1.11] as structure-directing agent for a niobia sol as described elsewhere (33). The O + oxide volume fraction in the as-made composite was 24%. After spin coating, composite thin films heat-treated to 130°C (33) were plasma-cleaned to remove the organics, resulting in the desired porous nanostructure. The nanopore lattice spacing as revealed by SEM data was 32.5 ± 8.5 nm; that is, similar to that of the aluminosilicate templates. Figure 3H displays SEM images of the resulting porous Si nanostructures after removal of the native oxide with dilute HF, a-Si deposition,

laser irradiation with two 40-ns laser pulses of 700 mJ/cm², and niobia template removal with concentrated HF (21). The images show that the Si has filled the pores, retaining the ~100-nm-thick template nanostructure. Cross-sectional TEM was performed on this sample to examine the epitaxial relationship between the silicon nanostructure and the substrate. In Fig. 3I, the Si lattice fringes in the porous nanostructure were found to be congruent with the fringes observed in the substrate, confirming the epitaxial correlation also for these thicker films (compare Fig. 3, A and I). Results of TEM imaging after plan-view polishing and electron diffraction on this sample are shown in fig. S1. Removing the native oxide layer before laser annealing indeed turned out to be a critical step. Fig. S2 shows two cross-sectional images of films etched for different times. When the native oxide was not removed completely (a 20-s etch), the crystal orientation in the film was independent of the substrate orientation and poly-Si formed. Extending the etch to 35 s resulted in single-crystal epitaxy in some regions of the film. In those regions where epitaxy had failed, the thin native oxide layer was still present. To go beyond the laboratory-scale proof of concept presented here, the oxide etchant needs to be able to penetrate small pores more reliably. This can be done with modern gas-phase semiconductor processing technology (with gaseous HF rather than liquid).

Finally, our bottom-up fabrication of epitaxially grown inorganic nanostructures was combined with top-down lithographic approaches to define specific areas, thus providing access to hierarchical nanostructures. As a proof-of-principle experiment, a TEM grid was used as a simple mask during the laser irradiation process to form patterns on a micrometer scale (Fig. 4E). The grid was placed in contact with the sample surface to minimize the loss of fidelity from the divergence of the homogenized incident laser beam (see fig. S3 for a schematic of the laser setup). The resulting Si pattern after irradiation through the TEM grid (Fig. 4F) indicates remarkable transfer. After template removal by HF, subsequent AFM imaging shows distinct squares of patterned material (Fig. 4G). Close-up images (Fig. 4H) of the square confirm the presence of Si nanostructures in the irradiated area and smooth flat Si in areas under the mask. As the image in Fig. 4G suggests and the AFM cross-sectional analysis confirms (Fig. 4H), the pattern edges of the irradiated areas are markedly higher than the interior of the areas. Despite poorly controlled edge effects, this simple proof-of-principle experiment does demonstrate that the placement of epitaxial single-crystal nanostructures can be controlled through simple imaging methods. Arbitrarily complex shapes should thus be possible to create by leveraging the wealth of patterning techniques currently available.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6001/214/DC1
Materials and Methods
Figs. S1 to S3
References

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An Oxidative Enzyme Boosting the Enzymatic Conversion of Recalcitrant Polysaccharides

Gustav Vaaje-Kolstad, Bjørge Westereng, Svein J. Horn, Zhanliang Liu, Hong Zhai, Morten Sørlie, Vincent G. H. Eijsink*

Efficient enzymatic conversion of crystalline polysaccharides is crucial for an economically and environmentally sustainable bioeconomy but remains unfavorably inefficient. We describe an enzyme that acts on the surface of crystalline chitin, where it introduces chain breaks and generates oxidized chain ends, thus promoting further degradation by chitinases. This enzymatic activity was discovered and further characterized by using mass spectrometry and chromatographic separation methods to detect oxidized products generated in the absence or presence of H₂¹⁸O or ¹⁸O₂. There are strong indications that similar enzymes exist that work on cellulose. Our findings not only demonstrate the existence of a hitherto unknown enzyme activity but also provide new avenues toward more efficient enzymatic conversion of biomass.

The transition to a more environment-friendly economy has spurred research on enzymes capable of efficiently degrading recalcitrant polysaccharides, such as cellulose and chitin (Fig. 1A), for the production of biofuels (1). Traditionally, enzyme systems capable of degrading such polysaccharides are considered to consist of endo-acting enzymes that cut randomly in the polysaccharide chain and processive exo-acting enzymes (chito- or cellobiohydrolases), which degrade the polymers from chain ends. All these enzymes are hydrolytic and are referred to as glycoside hydrolases. Although this model is generally accepted, it remains difficult to understand how the glycoside hydrolases could act on a poly-

saccharide chain in its crystalline environment, and biochemists have speculated about the existence of a substrate-disrupting factor that could make the crystalline substrate more accessible to hydrolytic enzymes (2).

Recently, it was discovered that microorganisms that break down chitin, a crystalline analog of cellulose occurring in the shells of insects and crustaceans, indeed produce a protein that increases substrate accessibility and potentiates hydrolytic enzymes (3) (Fig. 1B). These proteins are classified as carbohydrate-binding modules (CBMs) and belong to family CBM33 as defined in the Carbohydrate Active Enzymes (CAZy) database (4, 5). The first example of such a protein is CBP21 (CBP for chitin-binding protein), produced by the chitinolytic bacterium *Serratia marcescens*. As another example, two CBM33-containing proteins from *Thermobifida fusca* potentiate chitin hydrolysis by chitinases and cellulose hydrolysis by cellulases (6). Genes putatively encoding CBP21-like

proteins are abundant in bacteria and viruses but are rare in eukaryotes. Fungi produce proteins classified as family 61 glycoside hydrolases (GH61) that act synergistically with cellulases (7) and are structurally similar to CBM33 proteins (7, 8) (Fig. 1, B and C). The structural similarity includes a diagnostic conserved arrangement of the N-terminal amino group and two histidines that bind a metal ion (Fig. 1D). One of these histidines (His²⁸/His¹⁹ in Fig. 1D) is the N-terminal residue of the mature protein that results after proteolytic processing of the signal peptide during secretion. So far, the mechanisms employed by CBP21-like and GH61-like proteins have remained elusive.

We show here that CBP21 is an enzyme that catalyzes cleavage of glycosidic bonds in crystalline chitin, thus opening up the inaccessible polysaccharide material for hydrolysis by normal glycoside hydrolases. This enzymatic activity was first discovered when we detected traces of previously unidentified chitoooligosaccharides upon incubation of β -chitin nanowhiskers with CBP21 (Fig. 2A) (9). The products were identified as chitin oligosaccharides with a normal sugar at the nonreducing end and an oxidized sugar, 2-(acetylamino)-2-deoxy-D-gluconic acid (GlcNAcA), at the other end (Fig. 2B and fig. S1). Addition of reductants dramatically increased the efficiency of the reaction (Fig. 2, C and D), which enabled the breakdown of large crystalline β -chitin particles by CBP21 alone (Fig. 2), with the release of a range of oxidized products (figs. S2 and S3 and below). In the presence of a reductant such as ascorbic acid, CBP21 boosted chitinase efficiency to much higher levels than previously observed (Fig. 2D) (3). Biotechnological applications could take advantage of the ability to increase CBP21 activity by adjusting the reaction conditions.

If CBP21 acts randomly on crystalline surfaces, one would expect generation of longer

Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Post Office Box 5003, 1432 Ås, Norway.

*To whom correspondence should be addressed. E-mail: vincent.eijsink@umb.no

oligosaccharides, which are difficult to detect owing to their low solubility. The majority of soluble products generated by CBP21 in the presence of a reductant had a degree of polymerization (DP, i.e., the number of sugar moieties) below 10 (Fig. 2A and fig. S2). We therefore exploited a newly cloned chitin deacetylase from *Aspergillus nidulans* (AnCDA) (9) to increase the solubility of longer chitin fragments by deacetylation. This approach revealed the formation of chitin fragments with high DP, in the presence of CBP21 (Fig. 2E) or an endochitinase (ChiC) (Fig. 2F). Both CBP21 and ChiC generated long products, indicative of an “endo”-type of activity. Two important features stand out. First, all CBP21 products are oxidized, which confirms the observation that the cleavage of glycosidic bonds by CBP21 includes an oxidative step. Second, whereas the products released by ChiC represent a continuum of lengths (Fig. 2F), the products released by CBP21 are dominated by even-numbered oligosaccharides (Fig. 2E and figs. S2 to S4). Thus, ChiC tends to cleave any glycosidic bond, whereas CBP21 shows a strong preference for cleaving every second glycosidic bond. Keeping in mind the disaccharide periodicity in the substrate (Fig. 1A), this observation implies that ChiC approaches single polymer chains from “any side,” whereas CBP21 must approach the substrate from one side. The latter is consistent with polysaccharide cleavage in the context of an intact crystalline structure.

The CBP21-mediated cleavage mechanism was probed in more detail by isotope-labeling. Experiments in H_2^{18}O showed that one of the oxygen atoms introduced at the oxidized chain end comes from water (Fig. 3A). The only plausible source for the second oxygen was molecular oxygen, and this was confirmed by experiments performed in $^{18}\text{O}_2$ saturating conditions (Fig. 3, B and C). Removal of dissolved molecular oxygen in the reaction solution inhibited CBP21 activity (fig. S5), which confirmed the requirement for molecular oxygen for catalysis. Thus, the reaction catalyzed by CBP21 comprises a hydrolytic step and an oxidation step, as summarized in Fig. 3D. We suggest naming CBP21 a “chitin oxidohydrolase.”

CBP21 catalysis was found to be inhibited by EDTA, and activity could be restored by adding divalent cations such as Mg^{2+} or Zn^{2+} (fig. S6), which may bind to the conserved histidine motif (Fig. 1D). Note that the activity of GH61 proteins also depends on similar divalent cations (7). Structural studies of both CBP21 (10) and GH61 proteins (7, 8) show considerable structural plasticity in the metal-binding site, explaining why the metal-binding site is promiscuous and why the need for divalent cations is rather unspecific [as shown in fig. S6 and (7)]. Mutation of the second histidine (His¹¹⁴ in CBP21 and His⁸⁶ in GH61E from *Thielavia terrestris*) in the metal-binding motif knocked out activity of both proteins [fig. S7 and (7), respectively]. As CBP21 is a redox enzyme, it is remarkable that the metal ions activating CBP21 are not redox active. Perhaps the metal

ion's primary role lies in stabilization of the active-site structure and/or positioning of a hydrolytic water molecule.

Using newly developed methods for quantification of oxidized products that are described in detail in the supporting online material, we were able to estimate the speed and degree of oxidation under various conditions (fig. S8). When CBP21 acts alone on β -chitin under optimal conditions, the oxidation rate is on the order of 1/min, and the maximum extent of oxidation is about 7.6% of the sugars. Simultaneous degradation of β -chitin with CBP21 and ChiC under optimal conditions led to complete substrate solubilization and oxidation of ~4.9% of the sugars. It must be noted that, in nature, enzymes such as CBP21 normally act simultaneously with at least one, and in the case of *S. marcescens*, three chitinases (11).

Control experiments confirmed the conclusion that formation of oxidized products only occurs in the presence of CBP21 and crystalline substrates. The presence of reductants alone did not yield oxidized products (fig. S9) and did not potentiate or inhibit chitinase action (Fig. 2D and fig. S7). When incubated under optimal conditions with hexameric *N*-acetylglucosamine, neither degradation products nor oxidized oligosaccharides were observed (fig. S10). We also considered the

possibility that CBP21 might work without directly interacting with the cleaved bond, because one could envisage a CBP21-induced “destructuring” mechanism making the substrate more accessible for the action of reactive oxygen species generated in a CBP21-independent manner, e.g., by Fenton chemistry. However, we could not detect any soluble products after subjecting β -chitin to Fenton chemistry (fig. S11). All these experiments are consistent with CBP21's actively participating in the cleavage reaction and the oxidative step.

CBP21 activity is strongly inhibited by cyanide, a known O_2 mimic, but not by azide, a known inhibitor of heme proteins (fig. S5). Superoxide dismutase did not inhibit CBP21 activity, whereas catalase had only a minor inhibitory effect. Several reductants capable of functioning as electron donors boosted the activity of CBP21 (Fig. 2D and fig. S3). The experimental data indicate that the oxidation step catalyzed by CBP21 is cofactor-independent and depends on an external electron donor. The insensitivity for superoxide dismutase indicates that oxygen is activated on the enzyme, where it would be shielded from the solvent. The strong inhibition by cyanide supports the crucial role of the oxidative step. Cofactor-independent oxygenases have been described before, but these enzymes are normally thought to use conjugated

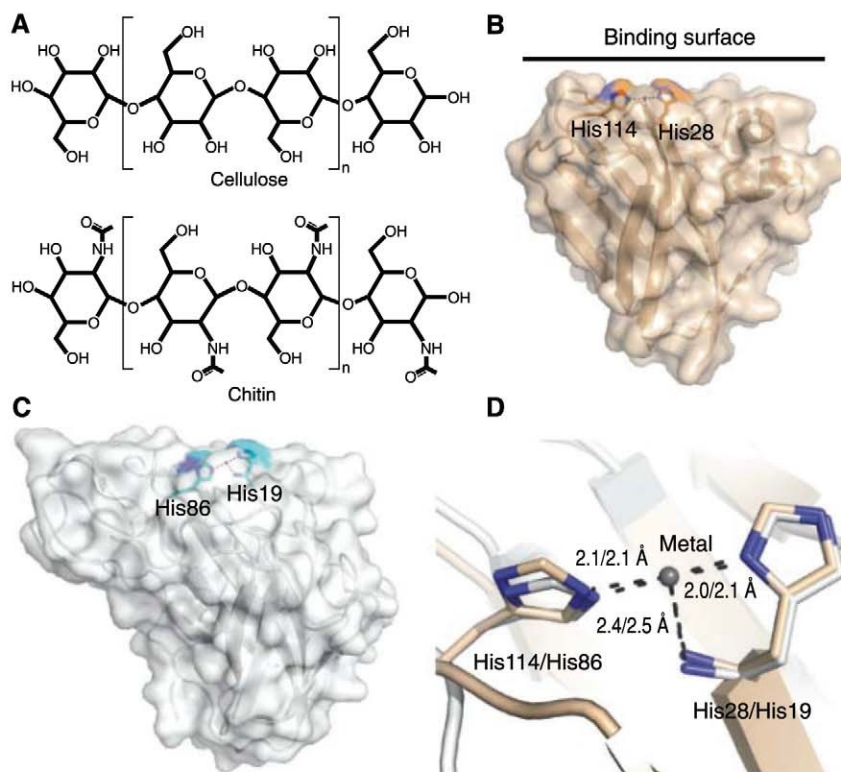


Fig. 1. (A) Repetitive disaccharide unit in cellulose and chitin. (B) Crystal structure of CBP21 (10) (PDB ID: 2BEM). The side chains of conserved histidine residues on the flat binding surface are shown in orange-colored stick representation. The binding surface has been identified previously using site-directed mutagenesis and binding studies (10) and is indicated by a black line. (C) Crystal structure of GH61E from *Thielavia terrestris* (7) (PDB ID: 3E1I). The side chains of conserved histidine residues are shown in cyan-colored stick representation. (D) Detailed view of the conserved arrangement of the two histidines and the N-terminal amino group in CBP21 (light brown) and GH61E (white), superimposed using PyMol. (B), (C), and (D) were made using PyMol.

Fig. 2. (A) Matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS) spectrum showing oxidized soluble chitooligosaccharides generated by CBP21 acting on β -chitin whiskers. (B) Oxidized chitooligosaccharide with a GlcNAc moiety. (C) Change in the polymeric material on treatment with CBP21 in the presence of ascorbic acid: 2.0 mg/ml β -chitin was treated with 1.0 μ M CBP21 in 20 mM Tris-HCl, pH 8.0, in the presence (left vial) or absence (right vial) of 1.0 mM ascorbic acid for 24 hours at 37°C. After the incubation, which leads to the oxidation of $\sim 7.6\%$ of the sugars (see main text), the glass vials were shaken vigorously. After a 1-min rest for particle sedimentation, the picture was taken. (D) Efficiency of β -chitin (0.45 mg/ml) degradation by 0.5 μ M ChiC in the presence of 1.0 μ M CBP21 and 1.0 mM ascorbic acid at pH 8.0 (magenta line on diamonds). Parallel reactions containing β -chitin and ChiC in the presence (red line on squares) or absence (yellow line on circles) of CBP21 (same concentrations as above) and without ascorbic acid are also shown. Data points for the incubation of β -chitin with ChiC and ascorbic acid overlap with the yellow points; this reaction is shown in fig. S7. Data are means $\pm$ SD ($n = 3$); error bars (not visible for every point) indicate SD. (E) MALDI-TOF MS spectrum of products obtained after incubating β -chitin (2.0 mg/ml) with 1.0 μ M AnCDA, 1.0 μ M CBP21, and 1.0 mM ascorbic acid for 16 hours, showing oxidized deacetylated chitooligosaccharides (all major products contain two acetylated sugars). A control reaction without CBP21 did not yield soluble products (results not shown). (F) MALDI-TOF MS spectrum of products obtained after incubating β -chitin (2.0 mg/ml) with 1.0 μ M AnCDA and 0.5 μ M ChiC for 8 hours, showing deacetylated chitooligosaccharides (all major products contain one acetylated sugar). MS peaks are labeled by observed atomic mass (listed in table S1) and the degree of polymerization (DP) of the oligosaccharide. Labels always refer to the peak of highest intensity in the respective cluster, which comprises several adducts; in (A) $[M+Na]^+$ ions are labeled; in (E) and (F), $[M+H]^+$ ions are labeled. “ox” indicates the presence of a GlcNAc moiety. Asterisks indicate a deacetylated product. In (A), (E), and (F), 100% relative intensity represents 2.5×10^4 , 1.1×10^3 , and 5.4×10^4 arbitrary units (a.u.), respectively. See fig. S1 for additional experiments to verify products and table S1 for an overview of possible ions and masses.

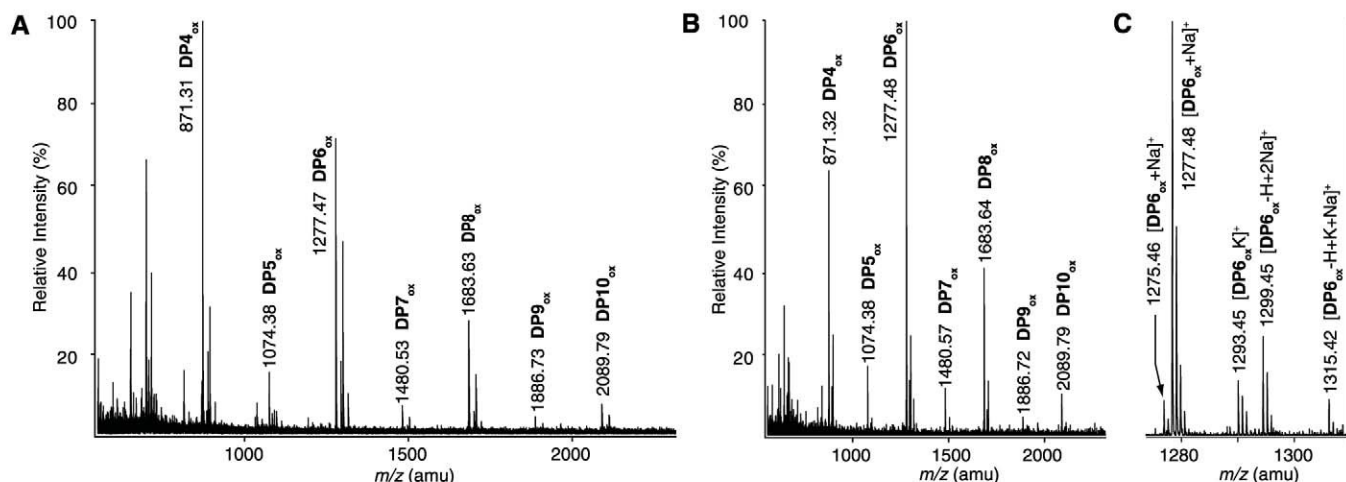
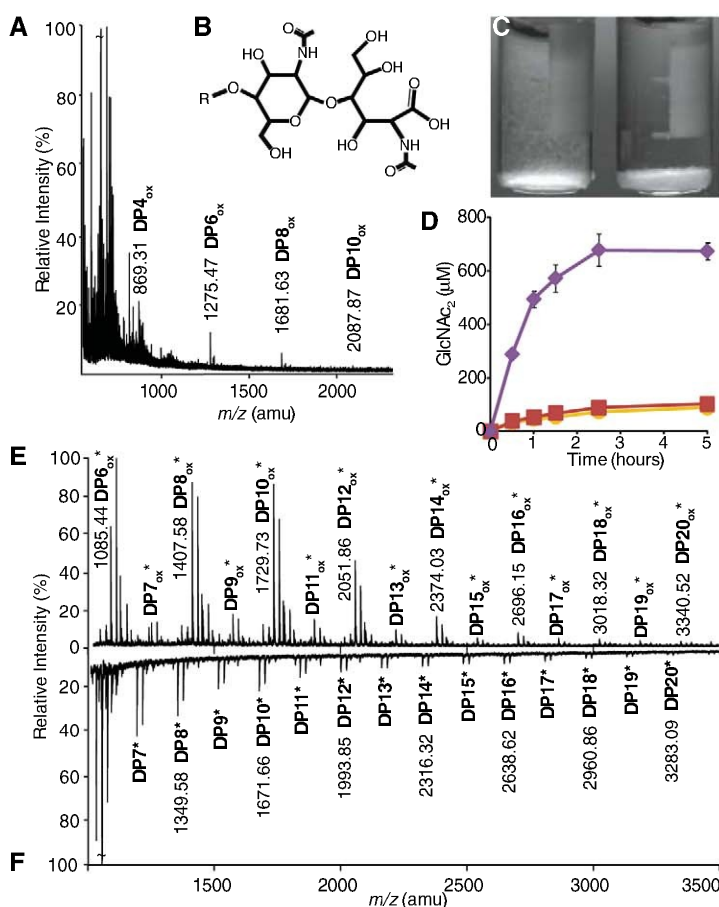


Fig. 3. MALDI-TOF MS analysis of products detected after treating 2.0 mg/ml β -chitin with 1.0 μ M CBP21 and 1.0 mM ascorbic acid in Tris-buffered $H_2^{18}O$ pH 8.0 (A) or in Tris-buffered $H_2^{16}O$ pH 8.0 (B). Labels in (A) and (B) refer to $[M+Na]^+$ ions (see table S1). All major products show a mass increase of 2 atomic mass units (amu) compared with reactions performed in solutions not containing isotope-labeled water or molecular oxygen (see Fig. 2A and fig. S2). (C) Adducts of the oxidized hexameric product shown in (B). Note the small amount of non-isotope-labeled product (indicated by the arrow), most likely resulting from the initial stage of the reaction where $^{16}O_2$ was still present (before $^{18}O_2$ saturation) (9). In (A) and (B), 100% relative intensity represents 6.8×10^3 and 2.0×10^3 a.u., respectively. (D) Scheme for the enzymatic reaction catalyzed by CBP21. In the final oxidized product, one oxygen comes from molecular oxygen (blue) and one from water (red).

carbanions in the substrates as electron donors (12), a mechanism that is not likely in the case of a polysaccharide substrate. If the oxidation step was to happen first, this would imply that CBP21 catalyzes cofactor-independent oxygenation of a saturated carbon, which is unprecedented and perhaps not very likely. On the other hand, such a mechanism could yield an intermediate product (for example, an ester bond) that may be more prone to hydrolysis than the original glycosidic bond. Alternatively, the hydrolytic step could occur first, which would imply that CBP21 is capable of hydrolyzing glycosidic bonds in a crystalline environment using a hitherto unknown mechanism. Such a hydrolytic step would require some degree of substrate distortion (13, 14), which seems challenging in a crystalline packing. However, in favor of this mechanism, the subsequent oxidation of the resulting sugar aldehyde ("reducing end") is more straightforward than oxidation of a saturated carbon. Clearly, further experiments are needed to unravel mechanistic details of the remarkable reaction catalyzed by CBP21.

CBP21 introduces chain breaks in what probably are the most inaccessible and rigid parts of crystalline polysaccharides, and its mode of action differs fundamentally from the mode of action of glycoside hydrolases. Glycoside hydrolases are designed to host a single "soluble" polysaccharide chain in their catalytic clefts, and their affinity and proximity to the crystalline substrate tend to be

mediated by nonhydrolytic binding domains. In contrast, CBP21 binds to the flat, solid, well-ordered surface of crystalline material and catalyzes chain breaks by a mechanism that results in oxidation of one of the new chain ends. The chain break will result in disruption of crystalline packing and increased substrate accessibility, an effect that may be enhanced by the oxidation of the new chain end that disrupts the normal chair conformation of the sugar ring and introduces a charge.

The enzyme activity demonstrated in this study is difficult to identify because products have low solubility and potentially a high tendency to remain attached to the crystalline material. Based on the structural homology and other similarities discussed above, we propose that GH61 proteins may have the same activity as CBP21, but the even lower product solubilities and higher crystalline packing of cellulose compared with chitin (15) make direct detection of this activity very challenging. However, a first glimpse of the potential of GH61 proteins for cellulose conversion has been presented recently (7). The dependency of these enzymes on the presence of molecular oxygen and reductants provides guidelines for process design.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6001/219/DC1
Materials and Methods
Figs. S1 to S12
Table S1
References

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Areawide Suppression of European Corn Borer with Bt Maize Reaps Savings to Non-Bt Maize Growers

W. D. Hutchison,^{1*} E. C. Burkness,¹ P. D. Mitchell,² R. D. Moon,¹ T. W. Leslie,³ S. J. Fleischer,⁴ M. Abrahamson,⁵ K. L. Hamilton,⁶ K. L. Steffey,^{7†} M. E. Gray,⁷ R. L. Hellmich,⁸ L. V. Kaster,⁹ T. E. Hunt,¹⁰ R. J. Wright,¹¹ K. Pecinovsky,¹² T. L. Rabaey,¹³ B. R. Flood,¹⁴ E. S. Raun^{15‡}

Transgenic maize engineered to express insecticidal proteins from the bacterium *Bacillus thuringiensis* (Bt) has become widely adopted in U.S. agriculture. In 2009, Bt maize was planted on more than 22.2 million hectares, constituting 63% of the U.S. crop. Using statistical analysis of per capita growth rate estimates, we found that areawide suppression of the primary pest *Ostrinia nubilalis* (European corn borer) is associated with Bt maize use. Cumulative benefits over 14 years are an estimated \$3.2 billion for maize growers in Illinois, Minnesota, and Wisconsin, with more than \$2.4 billion of this total accruing to non-Bt maize growers. Comparable estimates for Iowa and Nebraska are \$3.6 billion in total, with \$1.9 billion for non-Bt maize growers. These results affirm theoretical predictions of pest population suppression and highlight economic incentives for growers to maintain non-Bt maize refugia for sustainable insect resistance management.

During the past decade, adoption of transgenic crop technology increased worldwide to reach 134 million ha of transgenic crops planted in 25 countries during 2009 (1). In the United States, maize has been the most abundant transgenic crop planted to resist insect pests, with hybrids engineered to express insecticidal proteins isolated from the bacterium *Bacillus thuringiensis* [i.e., Bt maize (1, 2)]. Historically, the most widespread insect pest throughout the U.S. Corn Belt has been the European corn borer,

Ostrinia nubilalis (Hübner). The pest was accidentally introduced in the eastern United States in 1917 and subsequently spread with devastating results; losses are estimated at \$1 billion per year (3). Given the broad host range of *O. nubilalis*, the potential for Bt maize to suppress populations regionally was unclear. Furthermore, the economic impacts of such suppression had not been considered.

In 2009, plantings of Bt maize (with traits specific to preventing damage by lepidopteran

pests) reached 22.2 million ha, and for the first time exceeded 63% of the total area planted with maize in the United States (4). Most of the Bt maize is distributed throughout the Midwestern U.S. Corn Belt (4) (Fig. 1). Although "stacked" Bt events (maize varieties expressing multiple Bt toxins) directed at preventing herbivory from multiple insect pests are available (1, 4), nearly all Bt maize hybrids sold in the United States express toxins that control *O. nubilalis* (2, 4, 5). Because of Bt maize's high efficacy (6), there is concern that insects will evolve resistance to Bt

¹Department of Entomology, University of Minnesota, St. Paul, MN 55108, USA. ²Department of Agricultural and Applied Economics, University of Wisconsin, Madison, WI 53706, USA. ³Department of Biology, Long Island University, Brooklyn, NY 11201, USA. ⁴Department of Entomology, Pennsylvania State University, State College, PA 16802, USA. ⁵Minnesota Department of Agriculture, St. Paul, MN 55107, USA. ⁶Wisconsin Department of Agriculture, Trade and Consumer Protection, Madison, WI 53718, USA. ⁷Department of Crop Sciences, University of Illinois, Urbana, IL 61801, USA. ⁸USDA-ARS, Corn Insects and Crop Genetics Research Unit, Genetics Laboratory, Ames, IA 50011, USA. ⁹Syngenta Seeds Inc., Slater, IA 50244, USA. ¹⁰Department of Entomology, University of Nebraska, NEREC, Haskell Agricultural Laboratory, Concord, NE 68728, USA. ¹¹Department of Entomology, University of Nebraska, Lincoln, NE 68583, USA. ¹²Iowa State University, Nashua, IA 50658, USA. ¹³General Mills Inc., Le Sueur, MN 56058, USA. ¹⁴Del Monte Foods, Rochelle, IL 61068, USA. ¹⁵Pest Management Co., Lincoln, NE 68506, USA.

*To whom correspondence should be addressed. E-mail: hutch002@umn.edu

†Present address: Dow AgroSciences, Indianapolis, IN 46268, USA.

‡Deceased.

toxins (5, 7, 8). To delay evolution of resistance, the U.S. Environmental Protection Agency (EPA) mandated that a minimum 20 to 50% of total on-farm maize be planted as non-Bt maize within 0.8 km of Bt fields as a structured refuge for susceptible *O. nubilalis*. Use of non-Bt maize refugia is an important element of long-term insect resistance management (9).

Some maize producers have been skeptical of allowing *O. nubilalis* damage in non-Bt maize refugia (10, 11). However, modeling (7, 12) provided a theoretical rationale for how local suppression of *O. nubilalis* could occur. Suppression was supported by the hypothesis that preferential moth oviposition in early-planted Bt maize fields (7) would reduce larval damage in nearby late-planted non-Bt maize. More generally, for Bt and non-Bt maize fields with similar planting dates, *O. nubilalis* females are not able to distinguish between Bt and non-Bt maize for oviposition (13). Thus, with high larval mortality, Bt maize fields become an effective “dead-end” trap crop for *O. nubilalis* originating elsewhere (14). Although the models were theoretically appealing, it was not possible during early Bt maize commercialization to verify the magnitude of pest population suppression. Adult *O. nubilalis* are known to readily disperse among farms at distances of at least 800 m throughout their lifetime (15). Also, although maize is a major host, this pest colonizes >200 host plants including green beans, potato, and numerous weed species common to the Midwest region (3).

Surveys of *O. nubilalis* populations have extended from the initial documented invasion of the pest into the midwestern United States in the 1940s through the commercial adoption of Bt

maize during the period 1996 to 2009. Surveys have included statewide annual fall surveys (16) for diapausing larvae in Minnesota, Illinois, and Wisconsin, and less extensive summer trapping for adult moths with light traps (17, 18) in Illinois, Minnesota, Nebraska, and Iowa. These states have experienced a range of Bt maize adoption since 1996, including high levels in Minnesota, Nebraska, and Iowa, moderate levels in Illinois, and low levels in Wisconsin (Figs. 1 and 2) (18).

Historically, larval surveys have indicated that *O. nubilalis* populations have been episodic, characterized by ~6- to 8-year periodicity indicative of density-dependent population growth (7, 12). Much of the population cycling has been attributed to the pathogen *Nosema pyrausta* (12, 19). However, since commercialization of Bt maize, some periodicity has persisted (Fig. 2), but larval populations have declined relative to the pre-Bt era, particularly since 2002. These trends are evident in measures of larval abundance in non-Bt refuge fields alone, as well as in landscape-level means, for Bt- and non-Bt fields combined. Similar declines were found in measures of adult moth populations at eight locations in Minnesota, Illinois, Iowa, and Nebraska (18) (fig. S1).

To analyze the effects of Bt maize adoption on *O. nubilalis* populations, we estimated annual per capita growth rates (20) from fall larval surveys in non-Bt fields and analyzed them in relation to concurrent proportions of maize planted with Bt maize. Estimation also included antecedent larval densities in non-Bt fields, because *O. nubilalis* larval mortality increases with larval density (7, 12) and population growth more generally depends inversely on density (21). Analy-

sis used least-squares regression of growth rates in natural logarithm scale with three main effects: a state indicator variable to capture historical differences in mean densities among the three states, the natural logarithm of the antecedent larval density, and the proportion of Bt maize. Relative support for different models was evaluated with multimodel inference, with support weights based on the Bayesian information criterion, which balances reductions in residual sums of squares with numbers of parameters estimated (18, 22).

Relative support was greatest (82%) for the hypothesis that per capita growth rates differed among the three states, were inversely related to larval density, and were also inversely related to level of Bt maize adoption in each state (Table 1 and Fig. 3). The model with greatest support accounted for 38% of the variation in growth rates in non-Bt fields over all states and years combined. Models with just one or two of the three main effects and with interactions among the main effects had weak support (18) (table S2).

We used the fitted regression models to estimate mean densities for populations before and after adoption of Bt maize in each state (Table 1). Before Bt maize was adopted, the density in Minnesota was 59 larvae per 100 plants; from 1996 onward, when

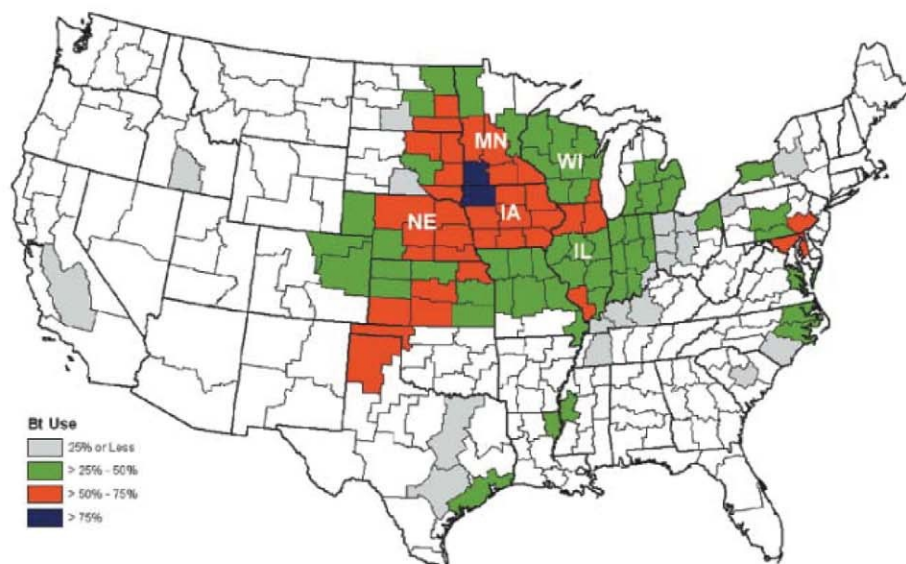


Fig. 1. Spatial distribution of maize containing one or more Bt traits for *O. nubilalis* control in 2006 in the United States. Bt maize data are from USDA crop reporting districts reporting >40,470 ha of maize, including the five states represented in this analysis (IL, Illinois; MN, Minnesota; WI, Wisconsin; IA, Iowa; NE, Nebraska). Areas in white had negligible maize hectares. Data are based on addresses of customer or retail outlet seed sales accounts, which may not accurately indicate cropping districts in which seed was ultimately planted. [©2008 Agricultural Biotechnology Stewardship Technical Committee]

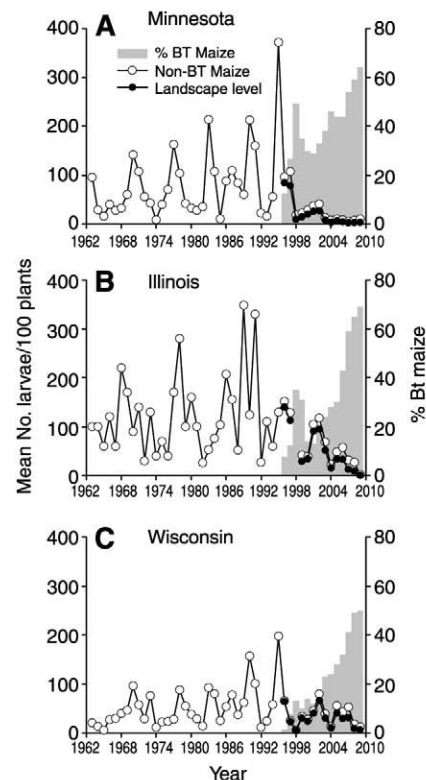


Fig. 2. Statewide average numbers of *O. nubilalis* larvae per 100 plants over the period 1963 to 2009 in (A) Minnesota, (B) Illinois, and (C) Wisconsin. Minnesota data were adjusted to landscape means (Bt and non-Bt maize fields) for comparisons with Illinois and Wisconsin landscape means, based on proportion of non-Bt corn hectares (18). Illinois and Wisconsin landscape means were adjusted for non-Bt maize hectares planted in each state (18).

Table 1. Regression statistics and estimated mean densities of *O. nubilalis* larvae per 100 plants before adoption of Bt maize in three midwestern states, and in non-Bt fields for 14 years (1996 to 2009) after adoption of Bt maize.

Coefficients for the regression model for per capita growth rate, $r = \ln(N_t/N_{t-1})$, are b_0 for intercept, b_1 for regressor $D = \ln(W_{t-1})$, and b_2 for regressor $PBt = \text{Bt maize proportion of crop}$.

Analysis*	State	n	R ²	Model coefficients			Pre-Bt density†		Avg. PBt	Bt-era density	
				b ₀ (±SE)	b ₁ (±SE)	b ₂ (±SE)	Mean	CI		Mean	CI
By state	Minnesota	46	0.35	2.75 (0.56)	−0.67 (0.13)	−2.20 (0.67)	59	40–88	0.40	16	9–29
	Illinois	64	0.44	4.35 (0.64)	−0.93 (0.14)	−2.98 (0.60)	105	87–128	0.32	38	26–56
	Wisconsin	67	0.37	2.82 (0.45)	−0.76 (0.12)	−1.10 (0.76)	40	31–51	0.23	29	19–44
Combined	Minnesota	—	—	3.07 (0.15)	—	—	57	44–75	0.40	18	11–27
	Illinois	177	0.38	3.51 (0.35)	−0.76 (0.07)	−2.23 (0.37)	103	80–131	0.32	40	28–57
	Wisconsin	—	—	2.85 (0.14)	—	—	43	32–58	0.23	22	15–31

Model fit to data from individual states separately, $r = b_0 + b_1D + b_2PBt$, or to the three states combined, but with differences among states reflected by state-specific intercepts. †Mean densities of larvae were estimated by setting $r = 0$ and solving for $N^ = \exp[-(b_0 + b_2PBt)/b_1]$ (see Fig. 3). Mean for pre-Bt era used $PBt = 0$; Bt era used 14-year average PBt . Confidence intervals (95% CIs) were estimated with the delta method (18) in log scale and then back-transformed to arithmetic scale.

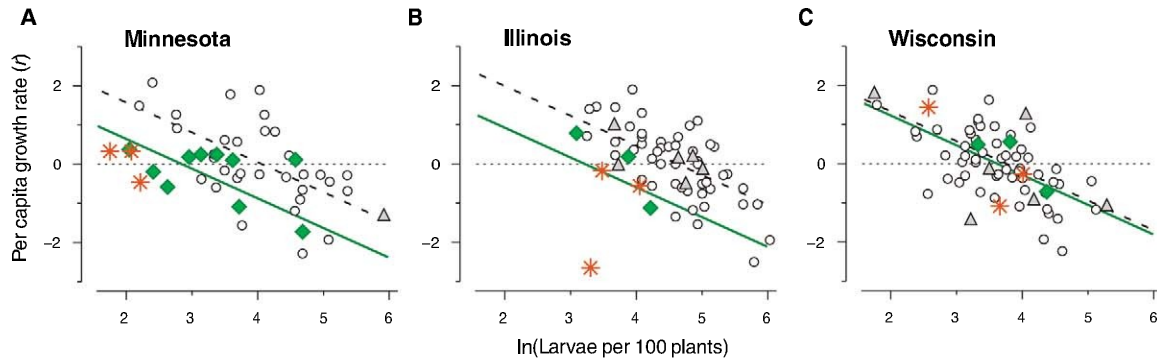


Fig. 3. Effects of Bt maize adoption on relation between larval density and annual per capita growth rates of *O. nubilalis* larval populations in non-Bt maize in three U.S. states: (A) Minnesota, (B) Illinois, (C) Wisconsin. Symbols indicate level of Bt maize adoption: open circles, pre-Bt years; gray triangles, 1 to 25%; green diamonds, 26 to 50%; orange asterisks, >51%. Bold dashed black line is least-

squares fit for main-effects model, states combined, with $PBt = 0$; green line is same with PBt equal to respective statewide 14-year average (Table 1). Intersections between dotted lines at $r = 0$ and bold dashed lines indicate estimated mean density before adoption of Bt maize, and intersections with green solid lines show extent to which density declined with adoption of Bt maize in each state (Table 1).

the proportion of maize planted to Bt averaged 0.40 (i.e., 40% adoption), mean density declined by ~73% to ~16 larvae per 100 plants. In Illinois and Wisconsin, where respective average Bt adoption levels were 32% and 23%, mean densities were reduced by ~64% and ~27%, respectively. Similar reductions in estimated mean densities were observed when data from all three states were analyzed together (Table 1) and when landscape-level means from Bt fields and non-Bt fields were analyzed (18) (table S3 and fig. S2). Although many factors are known to affect *O. nubilalis* population dynamics, including weather and natural enemies (3, 12, 16, 19), these results indicate that reductions in *O. nubilalis* were associated with commercialization of Bt maize.

Of the five states analyzed, Iowa, Illinois, Nebraska, and Minnesota are the top four maize-producing states in the United States, with yields in 2009 valued at \$27.1 billion (18) (tables S1 and S4). Combining analysis of the larval and moth data with annual USDA data for maize yield, price, and planted area, we estimated the annual benefits from 1996 to 2009 for both Bt- and non-Bt maize growers in each state (18). Direct benefits for Bt maize growers were calculated as the value of the yield gain for Bt maize relative to non-Bt maize, minus the additional cost for Bt maize seed (18) (tables S4 and S5). Suppression benefits for non-Bt maize growers

were calculated as the value of avoided yield losses under the assumption that the *O. nubilalis* populations in each state would have remained at their respective historical averages if Bt maize had not been commercialized. What actual *O. nubilalis* populations would have actually been without commercialization of Bt maize cannot be determined. However, midwestern farmers expected continual problems, as 67% of midwestern farmers reported in 1997 that *O. nubilalis* was a consistent problem in their fields (10). Mean yield losses for our analysis were calculated on the basis of *O. nubilalis* population densities and estimated models of larval stalk tunneling and associated yield loss (23, 24). Calculations used observed statewide survey densities for Illinois, Minnesota, and Wisconsin. For Iowa and Nebraska, observed average larval densities collected at research plots at locations around the state were used when available (1997, 2000, 2001, and 2002); otherwise, larval densities were estimated from historical averages at a few locations and the observed proportional larval decline in Minnesota, a state with Bt maize adoption rates similar to Iowa and Nebraska (18) (Fig. 1, table S1, and supplemental documentation file). Given the different nature of these larval data, loss estimates for Iowa and Nebraska are reported separately.

On the basis of these calculations, we estimate that cumulative benefits for both Bt and non-Bt

maize growers during the past 14 years were almost \$6.9 billion in the five-state region (18.7 million ha in 2009)—more than \$3.2 billion in Illinois, Minnesota, and Wisconsin, and \$3.6 billion in Iowa and Nebraska (Fig. 4). Of this \$6.9 billion total, cumulative suppression benefits to non-Bt maize growers resulting from *O. nubilalis* population suppression in non-Bt maize exceeded \$4.3 billion—more than \$2.4 billion in Illinois, Minnesota, and Wisconsin, and \$1.9 billion in Iowa and Nebraska—or about 63% of the total benefits. Direct benefits for Bt maize growers (Fig. 4, A and B) were reduced because of the additional cost for Bt seed over the 14 growing seasons, which we estimate to have a cumulative value of almost \$1.7 billion, whereas non-Bt maize experienced lower *O. nubilalis* damage as a result of areawide suppression at no additional cost.

In Illinois, Minnesota, and Wisconsin, suppression benefits for non-Bt maize growers (Fig. 4C) were initially larger (albeit dominated by Illinois and Minnesota) but more quickly exceeded the direct benefits for Bt maize, because population suppression occurred more rapidly than in Iowa and Nebraska (Fig. 4D). In Iowa and Nebraska, total grower benefits were larger because initial long-term population densities were greater. From 2007 onward, cumulative benefits for non-Bt maize growers exceeded benefits for Bt maize growers because suppression had become more effective.

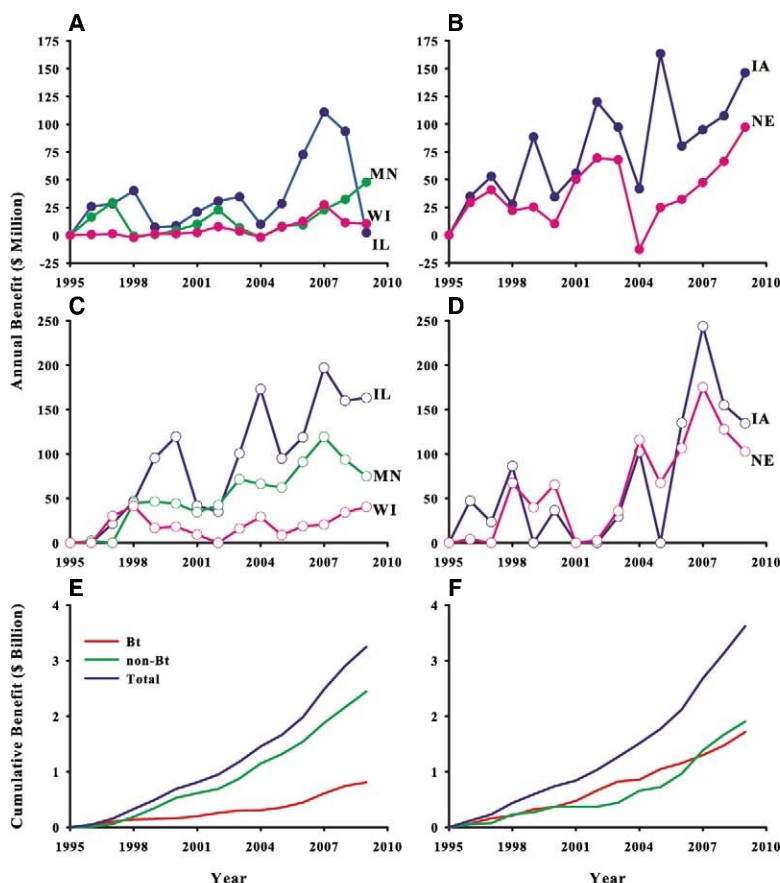


Fig. 4. (A and B) Annual benefits for Bt maize hectares, by state. (C and D) Annual pest suppression benefits for non-Bt hectares, by state. (E and F) Cumulative benefits across states. Benefits are expressed in 2009 dollars.

These benefit estimates do not incorporate effects of price changes and shifts in planted area that would have resulted without commercialization of Bt maize. Nevertheless, the calculations serve to indicate the potential magnitude of maize supply increase, and its market value resulting from area-wide suppression of *O. nubilalis* in these five states.

Regional reductions in the pink bollworm (*Pectinophora gossypiella*), which is fairly specialized to cotton (near-monophagous), have been reported from the use of Bt cotton in the United States (25). Also, areawide suppression of the polyphagous lepidopteran pest *Helicoverpa armigera* by Bt cotton in China has been reported (26). Reductions in *O. nubilalis* populations related to Bt maize have also been reported in other parts of the United States (27). We show here that pest suppression is directly associated with the use of transgenic maize. In addition, our findings indicate that economic benefits accrue not only to farmers planting Bt maize, but also to those planting non-Bt maize as a result of areawide pest suppression, and that these suppression benefits can equal or exceed the benefits to Bt maize growers.

These results highlight the need to account for economic benefits of pest suppression for non-Bt maize, as well as for direct economic benefits of Bt maize (28). Moreover, as *O. nubilalis* is highly poly-

phagous, the observed regional population declines suggest that traditional and organic farmers growing other crops might also benefit (29). Sustained economic and environmental benefits of this technology, however, will depend on continued stewardship by producers to maintain non-Bt maize refugia (5, 7–10) to minimize the risk of evolution of Bt resistance in crop pest species, and also on the dynamics of Bt resistance evolution at low pest densities and for variable pest phenotypes (30, 31).

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Supporting Online Material

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SOM Text
Tables S1 to S5
Figs. S1 and S2
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Freezing Tolerance in Plants Requires Lipid Remodeling at the Outer Chloroplast Membrane

Eric R. Moellering,^{1,2} Bagyalakshmi Muthan,¹ Christoph Benning^{1*}

Plants show complex adaptations to freezing that prevent cell damage caused by cellular dehydration. Lipid remodeling of cell membranes during dehydration is one critical mechanism countering loss of membrane integrity and cell death. *SENSITIVE TO FREEZING 2* (*SFR2*), a gene essential for freezing tolerance in *Arabidopsis*, encodes a galactolipid remodeling enzyme of the outer chloroplast envelope membrane. *SFR2* processively transfers galactosyl residues from the abundant monogalactolipid to different galactolipid acceptors, forming oligogalactolipids and diacylglycerol, which is further converted to triacylglycerol. The combined activity of *SFR2* and triacylglycerol-biosynthetic enzymes leads to the removal of monogalactolipids from the envelope membrane, changing the ratio of bilayer- to non-bilayer-forming membrane lipids. This *SFR2*-based mechanism compensates for changes in organelle volume and stabilizes membranes during freezing.

Freezing tolerance in plants is a critical factor limiting the geographic distribution of wild species. It also determines the agricultural range of important crops (e.g., citrus), because freezing damage in transitional climate zones can have devastating effects on agriculture. At the cellular level, freezing damage leads to leakiness of biomembranes enclosing organelles or the cell itself. Mechanistically, this is attributed to severe cellular dehydration initiated by extracellular ice formation that results in decreased water potential across the plasma membrane (1, 2). Under these conditions the formation of non-bilayer lipid structures, such as the inverted hexagonal II (H_{II})-type structures, can occur, and, thus, stabilization of lamellar membrane systems within the dehydrated plant cell is a key determining factor in the survival of freezing (1–3). To survive mild freezing, plants like *Arabidopsis* require preexposure to low, nonlethal temperatures in a process known as cold acclimation (2–4). Cold acclimation involves transcriptional (5) and metabolic (6) changes that result in multiple mechanisms protecting against freezing damage, including increases in intracellular solutes and the accumulation of cryoprotective metabolites (7) and proteins (8). However, the phenotype of *Arabidopsis* plants carrying mutations in the *SENSITIVE TO FREEZING 2* (*SFR2*) gene indicates a distinct mechanism required for freezing tolerance (9–11). The *sf2* mutants show extensive intracellular damage after freezing recovery, with rupture of both chloroplasts and tonoplasts, likely through fusion of destabilized membranes in these organelles. The *SFR2* protein is constitutively present, and *sf2* mutants are not affected in the regulation of known cold-acclimation processes (9–11).

With cellular membrane damage in mind, alterations in lipid composition during cold accli-

mation, such as increased fatty acid unsaturation and phospholipid content, have long been correlated with enhanced freezing tolerance but may be adaptations to growth at low above-freezing temperatures rather than freezing stress per se (1, 2). More recent lipid-profiling studies in *Arabidopsis* have revealed that more-drastic lipid compositional changes occur during freezing, including a decrease in the chloroplast-specific lipid monogalactosyldiacylglycerol (MGDG) (12, 13).

A link between *SFR2* and the reported freeze-dependent decrease in MGDG became evident in our effort to identify the enigmatic galactolipid: galactolipid galactosyltransferase (GGGT) (14). GGGT converts MGDG to di-, tri-, and tetragalactosyldiacylglycerol (DGDG, TGDG, and TeDG, respectively) by transglycosylation, resulting in the concomitant production of diacylglycerol (DAG) (15). The role of GGGT in galactolipid metabolism and the encoding gene(s) have remained unresolved since GGGT activity was first reported, but GGGT is known not to contribute significantly to DGDG synthesis in vivo during normal growth, as originally proposed (16, 17). In a reverse genetics candidate approach, we investigated new transfer-DNA mutant alleles disrupted in the *SFR2* gene (*sf2-3* and *sf2-4*, fig. S1) (18). *SFR2* is predicted to encode a glycosyl hydrolase family 1 (GH-1) protein for which the native substrates remain unknown. We considered *SFR2* to be a GGGT-encoding candidate because (i) some GH-1 family proteins are known to catalyze transglycosylation reactions similar to that postulated for GGGT (19, 20) and (ii) both the *SFR2* protein and GGGT activity have been unambiguously localized to the chloroplast outer envelope membrane (9, 21). Infiltration of wild-type leaves with $MgCl_2$ leads to TGDG and TeDG accumulation, presumably by activating GGGT. Applying this simple assay to the *sf2* mutants revealed their inability to accumulate these lipids, consistent with an apparent requirement of *SFR2* for GGGT activity in vivo (fig. S2).

The *sf2-3* and *sf2-4* alleles showed similar freezing sensitivity (Fig. 1A) as observed for pre-

viously described *sf2-1* and *sf2-2* alleles (11). Testing whether freezing could also induce GGGT, we observed that wild-type plants accumulated TGDG and TeDG during freezing with a corresponding ~20 mole percent (mol %) decrease in MGDG, whereas the *sf2* mutants did not (Fig. 1, B and C). Freezing treatment increased relative amounts of DGDG independent of *SFR2* (Fig. 1C). It seems possible that this increase is due to activity increases in the uridine 5'-diphosphate galactose (UDP-Gal)-dependent enzymes of DGDG biosynthesis after treatment or simply due to a relative decrease in other lipids classes. Expression of *SFR2* cDNA into *sf2-3* restored accumulation of TGDG and TeDG after $MgCl_2$ infiltration, indicating genetic complementation (fig. S3). We also found a ~7.5-fold increase in triacylglycerol (TAG) in wild-type freeze-treated plants, to 6% of total fatty acids esterified in TAGs (Fig. 1, D and E). Importantly, the *sf2* mutants showed ~50% reduction in TAG accumulation and a large decrease in 16:3 (number of carbons:number of double bonds) acyl groups esterified to TAG in freeze-treated plants compared with wild type (Fig. 1F). The presence of 16:3-containing TAG species in wild-type leaves was confirmed by mass spectrometry (fig. S4). The 16:3 acyl group is predominately found esterified to MGDG in *Arabidopsis* (22), indicating that DAG produced by GGGT activity is, in part, further acylated to TAG. DAG amounts did not change during freezing, although 16:3 was found specifically in freeze-treated wild-type plants (fig. S5). DAG derived from MGDG has also been shown to be converted to phosphatidic acid during freezing (12, 13). These results show that GGGT is activated as a result of freezing treatment, that the *sf2* mutants are defective in GGGT activation, and that DAG produced by GGGT during freezing is further metabolized to TAG, a nonpolar lipid not found in membranes.

The *SFR2* protein was produced in yeast by expression of the *SFR2* cDNA and localized to the isolated membrane fraction. Incubation of *SFR2*-containing membranes with deoxycholate-dispersed MGDG resulted in the production of DGDG, TGDG, and TeDG, whereas the same incubation of empty vector control membranes did not (Fig. 2A). In a time course using [³H] Gal-MGDG (23), *SFR2*-containing membranes converted ~60% of labeled MGDG into DGDG and oligogalactolipid products in 3 hours. This conversion was markedly reduced by the addition of EDTA equivalent to Mg^{2+} ions present (10 mM) (Fig. 2, B and C) as was previously observed in chloroplast envelopes (23). The fatty acids 16:3 and 18:3, specific to the plant-derived MGDG added to the assay, were present in the DAG pool only in the *SFR2* protein-containing reactions, indicating DAG is being produced from MGDG by *SFR2* in vitro. Proton-nuclear magnetic resonance (NMR) spectra of DGDG isolated from a scaled-up reaction showed the glycosidic linkages to be all in the β -anomeric configuration (β DGDG), which is distinct from the β ADGDG

¹Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824, USA. ²Department of Energy-Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA.

*To whom correspondence should be addressed. E-mail: benning@msu.edu

synthesized by the UDP-Gal-dependent DGD1/2 enzymes found in wild-type leaves during normal growth (15) (fig S6). The retention of configuration from β MGDG to β DGDG by SFR2 is consistent with all other GH-1 enzymes, which are classified as retaining β -glycosidases with a broad range of substrate specificities (24).

Many GH-1 family enzymes are known to have moderate transglycosidase activity under

certain in vitro conditions, and examples for which transglycosylation predominates over hydrolysis are known in other GH families (e.g., GH-70 glucanases) (20). SFR2, which is the most divergent among 253 GH-1 family proteins (25), has evolved to catalyze MGDG transgalactosylation (GGGT activity) by exclusion of water as an acceptor, which could be attributed to several intrinsic features of SFR2, including the

exclusive binding of acceptor galactolipids and/or the close apposition of the catalytic domain to the membrane surface. As such, SFR2 may provide insight into improving the transglycosidase activity of other GH-1 enzymes to produce oligosaccharides of medical or industrial importance (26). Orthologs of SFR2 are found in all land plants with completed genomes (9). Intriguingly, SFR2 is also conserved in species with no

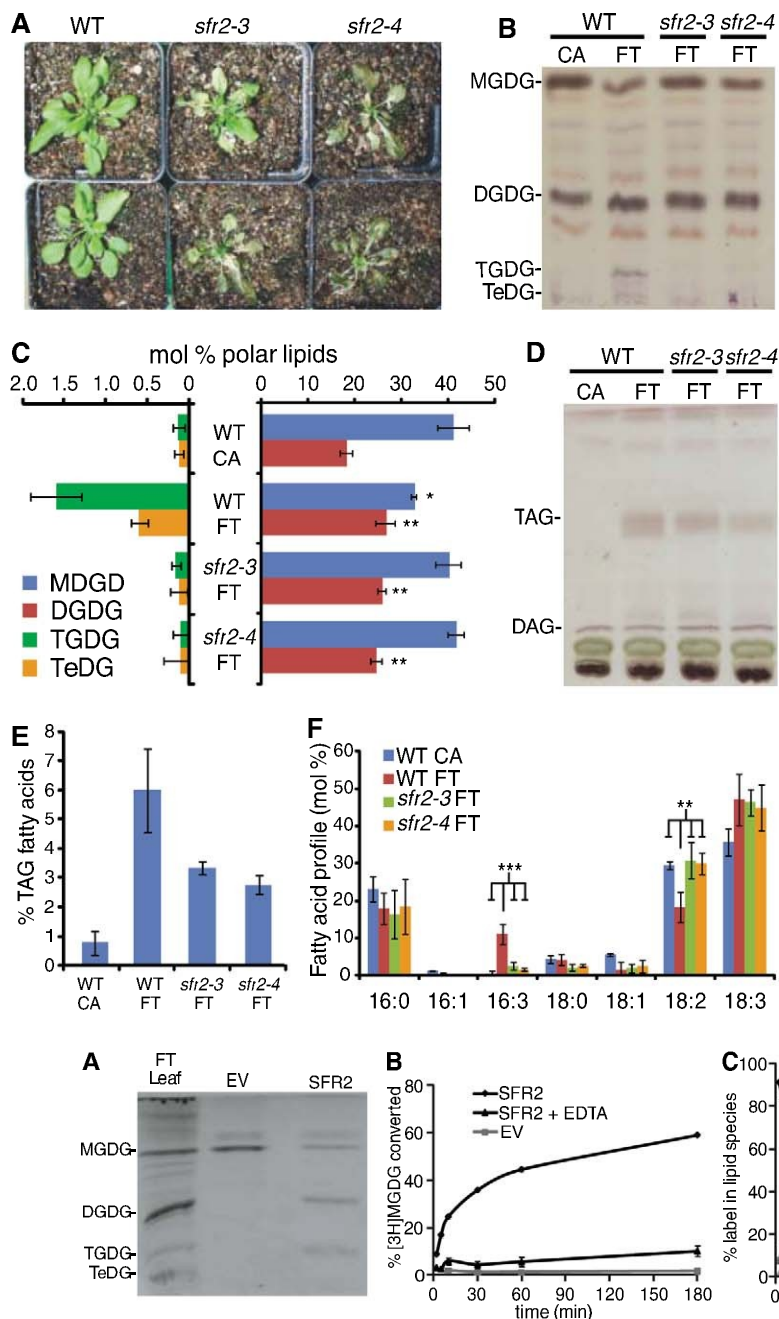


Fig. 1. Freeze-induced galactolipid remodeling is not observed in *sfr2* mutants. **(A)** Appearance of wild-type (Col2) and *sfr2* mutants at 5 days after freezing treatment. **(B)** Thin-layer chromatogram of lipid extracts stained for glycolipids with α -naphthol reagent from cold-acclimated (CA) or freeze-treated (FT) wild-type (WT) and *sfr2* plants. **(C)** Changes in galactolipid amounts in plants treated as in (B). * $P < 0.05$ or ** $P < 0.01$ versus WT CA levels for three biological repeats. Error bars indicate SD. **(D)** Thin-layer chromatogram of neutral lipids visualized by H_2SO_4 and charring from WT and *sfr2* plants treated as in (B). **(E)** Percent of total fatty acids esterified to TAG in plants treated as in (B); the average and standard deviation of at least three biological repeats are shown. **(F)** Fatty acid profile of TAGs plants treated as in (B) shown on a mol % basis of the average and standard deviation of at least three biological repeats; ** $P < 0.01$ or *** $P < 10^{-3}$ versus WT FT levels. The CA-treated control values for *sfr2-3* were 44.7 ± 2.0 mol % (average $\pm$ SD) MGDG and 18.7 ± 0.7 mol % DGDG and for *sfr2-4* were 43.9 ± 1.3 mol % MGDG and 17.9 ± 0.7 mol % DGDG. These were statistically not different from values for WT CA-treated plants shown in (C). The labels along the x axis indicate the fatty acid species identified.

Fig. 2. Recombinant SFR2 in vitro activity from transgenic yeast. **(A)** Thin-layer chromatogram of lipid extracts stained for galactolipids with α -naphthol reagent from in vitro GGGT activity assays using deoxycholate/MDG dispersions incubated with membrane fractions from empty vector control (EV) or SFR2 expression strain (SFR2). The in vitro reactions were stopped after 1 hour of incubation. Lipids from the reactions were extracted, chromatographed, and compared with lipids from a freeze-treated leaf sample (FT Leaf). **(B)** In vitro GGGT activity using labeled [3H]-Gal-MDG. The data are presented as the percent of

3H label found in DGDG, TGDG, and TeDG over time. Empty vector and SFR2 were assayed as in (A), and a SFR2 membrane protein assay containing EDTA at a concentration equimolar to Mg^{2+} present (10 mM) is also shown. Data are representative of two measurements, and the standard deviation is shown if it exceeds symbol size. **(C)** Percent of [3H] label found in different galactolipid species in the SFR2 assay shown in (B). **(D)** Fatty acid composition of diacylglycerol in empty vector control (EV) and SFR2 reactions at 3 hours. Data are presented on a mol % basis as the average and standard deviation of three biological repeats.

tolerance to freezing (e.g., tomato, maize, and rice), suggesting that SFR2 function is not restricted to freezing protection (9). There is a large overlap in the mechanisms required for freezing tolerance and dehydration because of water deficit or high salinity (3, 4), and SFR2 orthologs in these species may act on membrane stabilization during other abiotic stresses that cause cellular dehydration. Indeed, infiltration of *Arabidopsis* leaves with any osmotically active compound tested induced GGGT activity (table S1).

Our hypothesis for the requirement of SFR2-dependent galactolipid remodeling in freezing tolerance centers on the prevention of membrane fusion from the formation of non-bilayer H_{II}-type structures brought about by dehydration. During dehydration, non-bilayer structures are formed at the interface of apposed membranes and are believed to initiate at the chloroplast envelope membranes during freezing (1, 2, 8). This results in fusion between bilayers (27), particularly when membranes are enriched in glycerolipid species with relatively small head groups (e.g., MGDG and phosphatidylethanolamine), because these show a higher propensity for transition to the H_{II} phase in vitro. Previously, *sf2* mutants showed extensive chloroplast and tonoplast rupture in leaves during freezing recovery, which was proposed to arise from fusion of destabilized membranes (9). Here, we have shown that SFR2 partially converts MGDG to DGDG and oligogalactolipids, the latter of which are not prone to form H_{II} phases in vitro. In addition to the prevention of non-bilayer-type structures, accumulation of oligogalactolipids results in an increased average thickness of the head-group domain of the bilayers and an increase in the localized concen-

tration of hydroxyl groups per unit surface area, which enhance the repulsive hydration force between apposed bilayers during freeze-induced dehydration (28). Together, these factors could promote a sufficient distance between apposed bilayers that prevents membrane fusion. The overall phenomenon is analogous to the UDP-glucose-dependent modulation of mono- to diglycerolipid ratios observed in *Achelcplasma laidlawii* under different abiotic stress conditions (29). One distinction is that DAG is produced by SFR2 and is further metabolized to TAG and possibly other lipid species, thereby preventing the accumulation of DAG, which can form nonlamellar phases. In this regard, the action of SFR2 provides a mechanism by which polar membrane lipids and excess membrane are removed by conversion to nonpolar lipids (e.g., TAGs) to accommodate a shrinking organelle after freezing or, more generally, osmotic stress.

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Materials and Methods

Figs. S1 to S6

Table S1

References

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Frequent Mutations of Chromatin Remodeling Gene *ARID1A* in Ovarian Clear Cell Carcinoma

Siân Jones,¹ Tian-Li Wang,² le-Ming Shih,³ Tsui-Lien Mao,⁴ Kentaro Nakayama,⁵ Richard Roden,³ Ruth Glas,⁶ Dennis Slamon,⁶ Luis A. Diaz Jr.,¹ Bert Vogelstein,¹ Kenneth W. Kinzler,^{1*} Victor E. Velculescu,^{1*} Nickolas Papadopoulos^{1*}

Ovarian clear cell carcinoma (OCCC) is an aggressive human cancer that is generally resistant to therapy. To explore the genetic origin of OCCC, we determined the exomic sequences of eight tumors after immunoaffinity purification of cancer cells. Through comparative analyses of normal cells from the same patients, we identified four genes that were mutated in at least two tumors. *PIK3CA*, which encodes a subunit of phosphatidylinositol-3 kinase, and *KRAS*, which encodes a well-known oncoprotein, had previously been implicated in OCCC. The other two mutated genes were previously unknown to be involved in OCCC: *PPP2R1A* encodes a regulatory subunit of serine/threonine phosphatase 2, and *ARID1A* encodes adenine-thymine (AT)-rich interactive domain-containing protein 1A, which participates in chromatin remodeling. The nature and pattern of the mutations suggest that *PPP2R1A* functions as an oncogene and *ARID1A* as a tumor-suppressor gene. In a total of 42 OCCCs, 7% had mutations in *PPP2R1A* and 57% had mutations in *ARID1A*. These results suggest that aberrant chromatin remodeling contributes to the pathogenesis of OCCC.

Ovarian cancers are a heterogeneous group of diseases with distinct clinicopathological and molecular features (1). Among

them, OCCCs, which account for 10% of epithelial ovarian cancers, is one of the most aggressive types because, unlike the high grade-serous type,

it is refractory to standard platinum-based chemotherapy. Previous morphological and molecular studies have indicated that OCCC develops in a stepwise fashion from a common disease progenitor state, in many cases endometriosis, and then proceeds to frank malignancy (2–6). Activating mutations in *PIK3CA* (7) and genomic amplification of chr20q13.2 (8) are the most common molecular genetic alterations so far identified in OCCC.

To explore the genetic basis of this tumor type, we have determined the sequences of the ~18,000 protein-encoding genes listed in the RefSeq data-

¹Ludwig Center for Cancer Genetics and Therapeutics and Howard Hughes Medical Institute, Johns Hopkins Kimmel Cancer Center, Baltimore, MD 21231, USA. ²Department of Gynecology and Obstetrics and Oncology, Johns Hopkins Medical Institutes, Baltimore, MD 21231, USA. ³Department of Pathology, Oncology, Gynecology, and Obstetrics, Johns Hopkins Medical Institutes, Baltimore, MD 21231, USA. ⁴Department of Pathology, National Taiwan University College of Medicine, Taipei 100, Taiwan. ⁵Department of Gynecology and Obstetrics, Shimane University School of Medicine, Izumo, Shimane 6938501, Japan. ⁶Division of Hematology/Oncology, David Geffen School of Medicine at the University of California, Los Angeles, CA 90095, USA.

*To whom correspondence should be addressed. E-mail: npapado1@jhmi.edu (N.P.); kinzlike@jhmi.edu (K.W.K.); velculescu@jhmi.edu (V.E.V.)

base in tumors from eight patients (table S1). Because these tumors are composed of a mixture of cancer and stromal cells, we purified the cancer cells using epithelial cell target antibodies attached to magnetic beads (9). Staining of the cells bound to the beads revealed that >90% of them were OCCC cells. This procedure thereby maximized the sensitivity of the sequencing analyses by eliminating most of the contaminating normal cells (containing normal genomes) from the sample. DNA from the purified cells, as well as from normal cells obtained from the blood or uninvolved tissues of the same patients, were used to generate libraries suitable for massively parallel sequencing by synthesis (9). After capture of the coding sequences of the targeted genes with a SureSelect Enrichment System, the DNA was sequenced using an Illumina GAIIX platform. The average coverage of each base in the targeted regions was 84-fold, and 92.7% of these bases were represented in at least 10 reads (table S2).

Using stringent criteria for analysis of these data (9), we identified 268 somatic mutations in 253 genes among the eight tumors. The range of mutations per tumor was 13 to 125 alterations. Of these, 237 (88%) mutations were confirmed by Sanger sequencing (table S3). The tumor with 125 mutations (OCC06PT) was from a patient with recurrent disease that had previously been treated with chemotherapy. Excluding OCC06PT, there was an average of 20 mutations per tumor (tables S2 and S3). The mutation spectrum was

enriched for C to T transitions at 5'-CG base pairs, similar to those of other tumors whose exomes have been sequenced (10–14). Only four genes were mutated in more than one of the eight tumors studied: *PIK3CA*, *KRAS*, *FPP2R1A*, and *ARID1A*. The mutations in each of these four genes, and their somatic nature, were confirmed by Sanger sequencing of the DNA from the tumor and normal tissues of the corresponding patients (examples in Fig. 1). The sequences of these four genes were then determined in the tumor and normal tissues of an additional 34 OCCC cases using polymerase chain reaction amplification and Sanger sequencing with the primers listed in table S4. In total, *PIK3CA*, *KRAS*, *FPP2R1A*, and *ARID1A* mutations were identified in 40%, 4.7%, 7.1%, and 57% of the 42 tumors, respectively (Table 1).

Cancer cell lines with mutations in genes involved in cancer development provide valuable tools for further research. To this end, we extended the analysis of these four genes in seven OCCC cell lines that were derived from tumors independent of those described above. Five of the seven cell lines had mutations in *ARID1A* (nine mutations total), three had *FPP2R1A* mutations, one had a *KRAS* mutation, and four had *PIK3CA* mutations (table S5).

The nature of the somatic mutations in tumors can often be used to classify them as oncogenes or tumor suppressor genes (15). In particular, all bona fide oncogenes are mutated recurrently (that is, at the same codon or clustered at a few adja-

cent codons in different tumors), and the mutations are nearly always missense. In contrast, all bona fide tumor suppressor genes are mutated at a variety of positions throughout the coding region of the gene, and the mutations often truncate the encoded protein through production of a stop codon by a base substitution, an out-of-frame insertion or deletion (indel), or a splice site mutation. Moreover, tumor suppressor gene mutations generally affect both alleles, whereas mutations in oncogenes commonly affect only one allele.

Based on this logic, we can speculate about the likely function of the four genes in OCCC. *PIK3CA* and *KRAS* are well-studied oncogenes, and the 19 mutations identified in OCCC were heterozygous and clustered; fourteen of the 17 mutations in *PIK3CA* were at codons 542, 545, 546, or 1047, whereas both mutations in *KRAS* were at codon 12 (Table 1). The three mutations in *FPP2R1A* were similarly heterozygous and clustered, suggesting that it functions, when mutated, as an oncogene (Table 1). In contrast, the 32 mutations in *ARID1A* were distributed throughout the coding region, and all were predicted to truncate the protein through a base substitution resulting in a stop codon (9 mutations) or an out-of-frame insertion or deletion (23 mutations) (Table 1). In 11 of the 24 tumors with *ARID1A* mutations, both *ARID1A* alleles were affected through either a mutation in one allele and loss of heterozygosity of the other allele, or through two mutations that were presumably biallelic. Thus, we hypothesize that *ARID1A* functions as a tumor suppressor gene and that somatic mutations inactivate the gene product.

The serine/threonine protein phosphatase PP2A represents a family of holoenzymes with various activities. The holoenzyme contains a core composed of a heterodimer of a catalytic subunit (PPP2CA or PPP2CB) and a constant regulatory subunit (PPP2R1A or PPP2R1B). PPP2R1A serves as a scaffold to coordinate the interaction of the core enzyme with one of more than 15 regulatory subunits to form the heterotrimeric holoenzyme (16, 17). Somatic mutations in *FPP2R1A* are not listed in the Cancer Gene Census of the Catalogue of Somatic Mutations in Cancer (COSMIC) database, although a few alterations in this gene have been previously reported (18). Functional studies have shown that PP2A is involved in the control of cell growth and division. Specifically, this protein is required for proper chromosome segregation through its interactions with Bub1 and Sgo1 (19). The two arginine residues that were somatically mutated in OCCC are highly conserved and reside within one of the Huntington, elongation factor3, PP2A, TOR (HEAT) domains of PPP2R1A that are involved in binding regulatory subunits.

The protein encoded by *ARID1A* can bind to AT-rich DNA sequences and is a component of the adenosine triphosphate-dependent chromatin modeling complex switch/sucrose-nonfermentable (SWI/SNF). The SWI/SNF chromatin-remodeling complex mobilizes nucleosomes and functions as

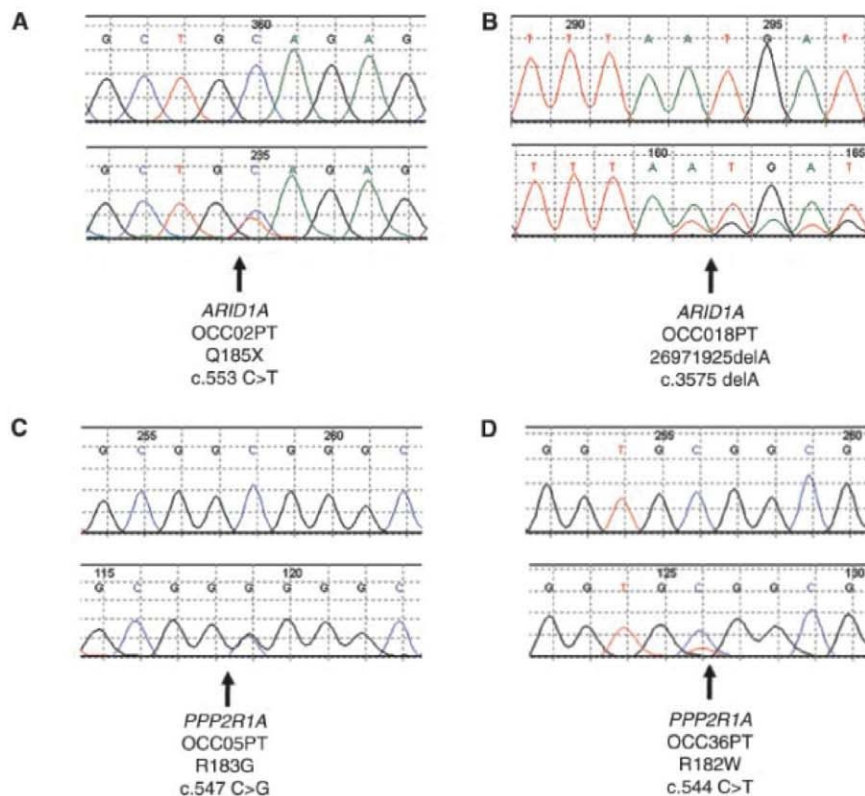


Fig. 1. Sequence chromatograms showing somatic *ARID1A* and *PPP2R1A* mutations. The lower panels (C and D) show the tumor, and the upper panels (A and B) show the matched normal control.

Table 1. Mutations in *ARID1A*, *KRAS*, *PIK3CA*, and *PPP2R1A* in human ovarian clear cell carcinomas.*

Sample†	Gene	Transcript accession	Nucleotide (genomic)‡	Nucleotide (cDNA)	Amino acid (protein)	Mutation type
OCC01PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26972561_26972562insA	c.3854_3855insA	fs	Indel
OCC02PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26896034C>T	c.553C>T	p.Q185X	Nonsense
OCC02PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26978879-26978880dupGT	c.5903_5904dupGT	fs	Indel
			g.chr1:26972009_26972034delTGATGGGGCG	c.3659_3684delTGATGGGGCG		
OCC03PT	<i>ARID1A</i>	CCDS285.1	CATGTCCTATGAGCCA (hom)	CATGTCCTATGAGCCA	fs	Indel
OCC07PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26896066C>A	c.585C>A	p.Y195X	Nonsense
OCC08PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26970389delC	c.3391delC	fs	Indel
OCC10PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26972790_26972792dupGCA (hom)	c.4001_4002dupGCA (hom)	fs	Indel
OCC10PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26979804_26979805delTG (hom)	c.6828_6829delTG (hom)	fs	Indel
OCC11PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26930334_26930335insCCTAC	c.1451_1455insCCTAC	fs	Indel
OCC13PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26974233_26974234insTGGC	c.4926_4927insTGGC	fs	Indel
OCC14PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26972886_26972887_delTT (hom)	c.4011_4012delTT (hom)	fs	Indel
OCC15PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26973940G>A	c.4635G>A	p.W1545X	Nonsense
OCC15PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26978178T>A	c.5202T>A	p.Y1734X	Nonsense
OCC16PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26895967_26895973delCGCCGCC (hom)	c.486_492delCGCCGCC (hom)	fs	Indel
OCC18PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26971925delA	c.3575delA	fs	Indel
OCC20PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26970221delG	c.3223delG	fs	Indel
OCC22PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26979694dupG	c.6718dupG	fs	Indel
OCC23PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26896379_2689637980_insCGTC	c.898_899insCGTC	fs	Indel
OCC23PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26979686_26979687insT	c.6710_6711insT	fs	Indel
OCC24PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26930542C>T	c.1663C>T	p.Q555X	Nonsense
OCC27PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26896263_26896272delCGTCGCTTC	c.782_791delCGTCGCTTC	fs	Indel
OCC27PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26971984_26971994delCAGCCCAGTAT	c.3634_3644delCAGCCCAGTAT	fs	Indel
OCC30PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26931823C>T	c.1873C>T	p.Q625X	Nonsense
OCC32PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26960135C>T	c.2122C>T	p.Q708X	Nonsense
OCC34PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26931754G>T	c.1804G>T	p.E602X	Nonsense
OCC34PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26979678delT	c.6702delT	fs	Indel
OCC36PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26928932T>G	c.1341T>G	p.Y447X	Nonsense
OCC36PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26971613delC	c.3442delC	fs	Indel
OCC39PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26896364dupC	c.883dupC	fs	Indel
OCC39PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26965434delC	c.2868delC	fs	Indel
OCC41PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26931831delT	c.1881delT	fs	Indel
OCC42PT	<i>ARID1A</i>	CCDS285.1	g.chr1:26960479_26960488delCGGCCACCCA	c.2179_2188delCGGCCACCCA	fs	Indel
OCC04PT	<i>KRAS</i>	CCDS8703.1	g.chr12:25289551C>T	c.35G>A	p.G12D	Missense
OCC05PT	<i>KRAS</i>	CCDS8703.1	g.chr12:25289551C>G	c.35G>C	p.G12A	Missense
OCC01PT	<i>PIK3CA</i>	CCDS43171.1	g.chr3:180418788C>A	c.1636C>A	p.Q546K	Missense
OCC02PT	<i>PIK3CA</i>	CCDS43171.1	g.chr3:180418776G>A	c.1624G>A	p.E542K	Missense
OCC06PT	<i>PIK3CA</i>	CCDS43171.1	g.chr3:180418785G>A	c.1633G>A	p.E545K	Missense
OCC08PT	<i>PIK3CA</i>	CCDS43171.1	g.chr3:180418785G>A	c.1633G>A	p.E545K	Missense
OCC09PT	<i>PIK3CA</i>	CCDS43171.1	g.chr3:180434779A>T	c.3140A>T	p.H1047L	Missense
OCC10PT	<i>PIK3CA</i>	CCDS43171.1	g.chr3:180434779A>G	c.3140A>G	p.H1047R	Missense
OCC11PT	<i>PIK3CA</i>	CCDS43171.1	g.chr3:180418777A>T	c.1625A>T	p.E542V	Missense
OCC13PT	<i>PIK3CA</i>	CCDS43171.1	g.chr3:180434779A>G	c.3140A>G	p.H1047R	Missense
OCC15PT	<i>PIK3CA</i>	CCDS43171.1	g.chr3:180410152C>G	c.1221C>G	p.C407W	Missense
OCC20PT	<i>PIK3CA</i>	CCDS43171.1	g.chr3:180434779A>G	c.3140A>G	p.H1047R	Missense
OCC22PT	<i>PIK3CA</i>	CCDS43171.1	g.chr3:180434779A>G	c.3140A>G	p.H1047R	Missense
OCC23PT	<i>PIK3CA</i>	CCDS43171.1	g.chr3:180399648_180399649insCCTCAA	c.341_342insCCTCAA	N114_R115insLN	Indel
OCC27PT	<i>PIK3CA</i>	CCDS43171.1	g.chr3:180399638A>G	c.331A>G	p.K111E	Missense
OCC30PT	<i>PIK3CA</i>	CCDS43171.1	g.chr3:180434779A>G	c.3140A>G	p.H1047R	Missense
OCC35PT	<i>PIK3CA</i>	CCDS43171.1	g.chr3:180418776G>A	c.1624G>A	p.E542K	Missense
OCC36PT	<i>PIK3CA</i>	CCDS43171.1	g.chr3:180418785G>A	c.1633G>A	p.E545K	Missense
OCC42PT	<i>PIK3CA</i>	CCDS43171.1	g.chr3:180434779A>G	c.3140A>G	p.H1047R	Missense
OCC05PT	<i>PPP2R1A</i>	CCDS12849.1	g.chr19:57407794C>G	c.547C>G	p.R183G	Missense
OCC07PT	<i>PPP2R1A</i>	CCDS12849.1	g.chr19:57407794C>T	c.547C>T	p.R183W	Missense
OCC36PT	<i>PPP2R1A</i>	CCDS12849.1	g.chr19:57407791C>T	c.544C>T	p.R182W	Missense

*Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. †Samples OCC01 to OCC08 were used for the initial (discovery) screen for mutations. ‡Coordinates refer to the human reference genome hg18 release (NCBI 36.1, March 2006).

a regulator of gene expression and chromatin dynamics. ARID1A is one of the two mutually exclusive ARID1 subunits of the SWI/SNF complex and is thought to provide specificity to this complex (20). Changes in chromatin can influence the epigenetic regulation of many genes, inducing those that play a role in cancer (20–22). Indeed, functional studies have implicated ARID1A in the ability of the SWI/SNF complex to inhibit cell growth (23). No mutations of *ARID1A* are listed in the Cancer Gene Census of the COSMIC database, but chromosomal translocations that involve this gene have been identified in a human breast cancer and a human lung cancer cell line (24). Knock-down of ARID1A in a leukemia cell line confers resistance to Fas-mediated apoptosis (25).

The results of this study emphasize two themes in modern cancer genetics. The first is that specific tumor types are characterized by mutations in “communal cancer genes” like *KRAS* and *PIK3CA*, as well as in “restricted cancer genes” like *PPP2R1A* and *ARID1A*. The communal cancer genes are involved in a variety of cancers and have been extensively studied. Restricted cancer genes have been shown to contribute to specific types of leukemias and sarcomas, mainly through translocations (e.g., the *ABL* oncogene in chronic myelogenous leukemia and *EWS* fused to a gene encoding an ETS transcription factor family member in Ewing’s sarcoma). With the advent of whole exome sequencing, we are beginning to see similar specificity with respect to point mutations [e.g., *IDH1* in gliomas (14) and *GNAQ* in uveal melanomas (26)].

The second theme is that mutations of chromatin-modifying genes are characteristic of certain tumor types. Recent examples include the *ARID1C* gene, also known as lysine (K)-specific demethylase 5C (*KDM5C*), in renal cell cancers (27), *SMARCA4/BRG1* (SWI/SNF related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 4) in lung cancers (28), and now *ARID1A* in OCCC. Epigenetic changes in cancers, including methylation of deoxycytidine residues in DNA and a variety of covalent modifications of chromatin proteins, have been extensively studied (20–22, 29). Interestingly, however, the reason(s) that DNA methylation and chromatin are different in cancer cells than in normal cells is completely unknown. Similarly, the relationship between the genetic alterations that unequivocally drive tumorigenesis and the epigenetic changes that are so widespread in tumor genomes has not been defined. Discovery of tumor suppressor genes such as *ARID1A* that are mutated in cancers bridge this gap, because they are likely to directly lead to epigenetic changes in cancer cells through specific modifications of chromatin proteins. They additionally provide a potential approach to determine which of the numerous epigenetic changes in cancers confer a selective growth advantage and which are simply “passengers” that do not play a causal role. The identification of the genes whose expression is

specifically modulated by *ARID1A* inactivation will be the next crucial step in this line of research.

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Supporting Online Material

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Histone H3 Thr-3 Phosphorylation by Haspin Positions Aurora B at Centromeres in Mitosis

Fangwei Wang,¹ Jun Dai,¹ John R. Daum,² Ewa Niedzialkowska,³ Budhaditya Banerjee,³ P. Todd Stukenberg,³ Gary J. Gorbisky,² Jonathan M. G. Higgins^{1*}

Aurora B is a component of the chromosomal passenger complex (CPC) required for correct spindle-kinetochore attachments during chromosome segregation and for cytokinesis. The chromatin factors that recruit the CPC to centromeres are unknown, however. Here we show that phosphorylation of histone H3 threonine 3 (H3T3ph) by Haspin is necessary for CPC accumulation at centromeres and that the CPC subunit Survivin binds directly to H3T3ph. A nonbinding Survivin-D70A/D71A mutant does not support centromeric CPC concentration, and both Haspin depletion and Survivin-D70A/D71A mutation diminish centromere localization of the kinesin MCAK and the mitotic checkpoint response to taxol. Survivin-D70A/D71A mutation and microinjection of H3T3ph-specific antibody both compromise centromeric Aurora B functions but do not prevent cytokinesis. Therefore, H3T3ph generated by Haspin positions the CPC at centromeres to regulate selected targets of Aurora B during mitosis.

The chromosomal passenger complex (CPC)—which contains Aurora B, inner centromere protein (INCENP), Survivin, and Borealin—is found on chromosome arms in prophase, concentrates at inner centromeres during prometaphase, and transfers to the central spindle at anaphase (1). Aurora B phosphorylates several substrates at these locations, including histone H3 Ser¹⁰ (H3S10ph) on chromosome arms, mitotic centromere-associated kinesin (MCAK) at inner centromeres, centromere protein-A Ser⁷ (CENP-

AS7ph) at outer centromeres, and the KNL1/Mis12 complex/Ndc80 network at kinetochores (1–7). Current models suggest that centromeric Aurora B responds to lack of tension across sister kinetochores that are incorrectly attached to the spindle. Bipolar kinetochore attachment forces may pull kinetochore substrates away from inner centromeric Aurora B, which would lead to substrate dephosphorylation, selective stabilization of correct microtubule attachments, and eventually satisfaction of the spindle checkpoint (fig. S1) (8).

Despite its central importance, it is not understood how Aurora B accumulates at centromeres (9–11).

Immunofluorescence microscopy of mitotic cells shows that phosphorylated histone H3 Thr³ (H3T3ph) and Aurora B localize similarly at inner centromeres (Fig. 1A and fig. S2) (12). We therefore tested whether Haspin, which is responsible for generating H3T3ph in mitosis (13, 14), is required for CPC localization. When Haspin was subjected to RNA interference (RNAi) in HeLa cells, a marked reduction in Aurora B at centromeres (greater than fivefold) and an increase on chromosome arms were observed (Fig. 1, B and C). In contrast, Haspin depletion had little effect on the amounts of CPC subunits in mitosis and did not disrupt CPC formation (fig. S3). Similar

results were obtained in a human osteosarcoma cell line (U2OS cells), where Haspin RNAi delocalized all CPC components from centromeres (Fig. 2A and fig. S4). Microinjection of H3T3ph-specific antibody into mitotic LLC-PK pig kidney cells caused a similar delocalization of Aurora B (Fig. 1, D and E), indicating that Haspin acts through H3T3ph to position the CPC during mitosis and not through effects at other cell cycle stages.

To determine whether the reduced CPC accumulation at centromeres after Haspin depletion was functionally significant, we first focused on MCAK, a microtubule-depolymerizing kinesin whose inner centromeric localization is dependent on Aurora B (3, 4, 15). Both Aurora B and Haspin RNAi reduced the centromeric enrichment of MCAK in $\geq 70\%$ of U2OS cells (Fig. 2, B to D) and HeLa cells (fig. S5). Artificial retargeting of Aurora B to centromeres with CENP-B-INCENP (8) largely restored MCAK localization in Haspin-depleted cells (fig. S6). Disruption of CPC activity also compromises the spindle checkpoint response, particularly to low doses of taxol (16–20). Consistent with this, HeLa cells transfected with Haspin small interfering RNA (siRNA) were less efficiently arrested in mitosis by taxol than controls

were (Fig. 2E). Thus, Haspin appears to influence MCAK localization and checkpoint signaling at centromeres by controlling Aurora B localization.

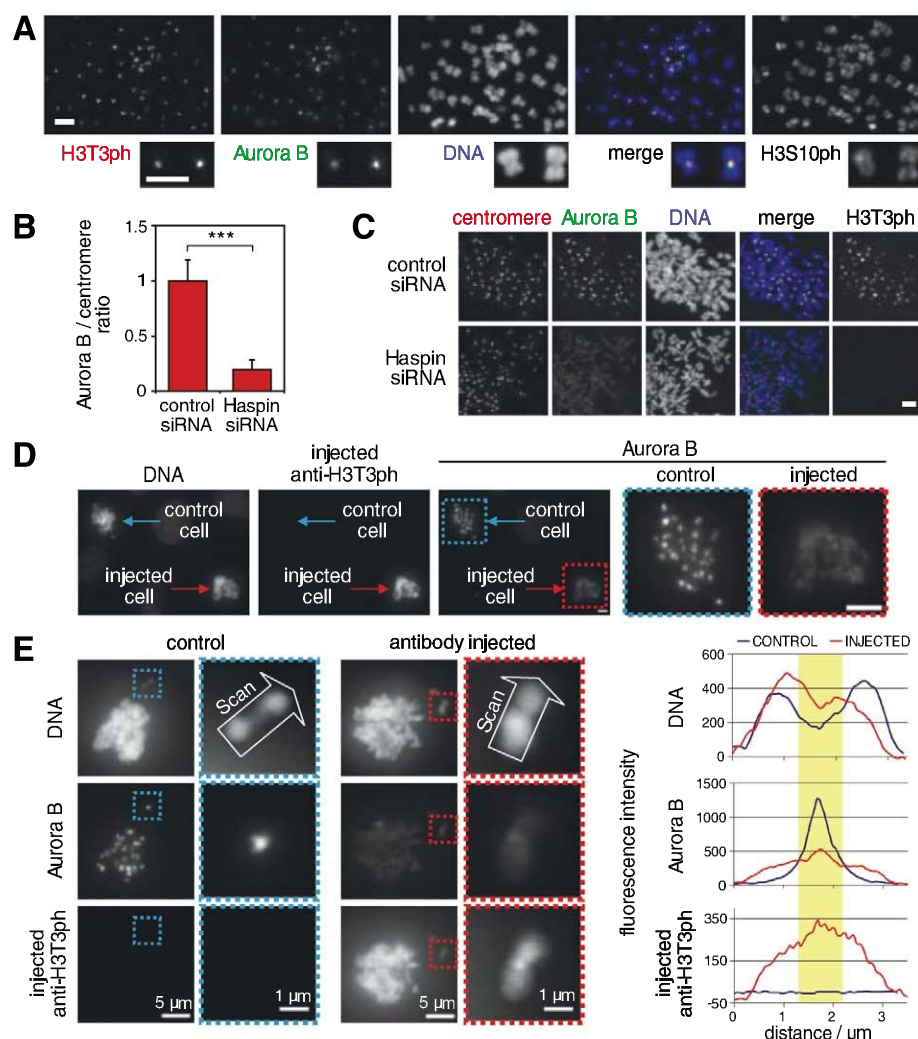
Two experiments suggest that these effects are not a consequence of defects in chromosome cohesion caused by Haspin RNAi (14). First, Haspin overexpression, which increases cohesion between chromosome arms (14), also influenced Aurora B localization (fig. S7). Second, loss of cohesion induced by depletion of Sgo1 (14, 21, 22) rendered Aurora B localization more diffuse, but it remained enriched at centromeres in $>80\%$ of nocodazole-treated cells, and centromeric MCAK was little changed (Fig. 2, A to D). Therefore, as reported elsewhere (14, 21–24), loss of centromeric CPC and MCAK is not an inevitable consequence of cohesion loss.

In contrast, Haspin RNAi had minor effects on total CENP-AS7ph and H3S10ph (fig. S8A), although both were reduced by Aurora B RNAi, as expected (2, 5, 16). Haspin RNAi also did not detectably alter total Thr²³² autophosphorylation (fig. S8B) or *in vitro* kinase activity of immunoprecipitated Aurora B (fig. S8C). H3S10ph, CENP-AS7ph, and Aurora B T232ph immunofluorescence remained present in individual U2OS

¹Division of Rheumatology, Immunology, and Allergy, Brigham and Women's Hospital, Harvard Medical School, Smith Building, 1 Jimmy Fund Way, Boston, MA 02115, USA. ²Cell Cycle and Cancer Biology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104, USA. ³Department of Biochemistry and Molecular Genetics, University of Virginia School of Medicine, Charlottesville, VA 22908, USA.

*To whom correspondence should be addressed. E-mail: jhiggins@rics.bwh.harvard.edu

Fig. 1. Haspin RNAi or microinjection of H3T3ph-specific antibody delocalizes Aurora B from mitotic centromeres. **(A)** Immunofluorescence microscopy of nocodazole-arrested HeLa chromosome spreads reveals colocalization of H3T3ph and Aurora B. **(B and C)** Cells were transfected with siRNA and prepared as in (A). The Aurora B/centromere autoantigen intensity ratio was determined by immunofluorescence at ~ 40 centromeres in nine cells per condition (B). Means $\pm$ SD are shown ($n = 3$). *** $P < 0.001$ by Student's *t* test. Example images are shown in (C). **(D and E)** Nocodazole- and MG132-treated LLC-PK cells were microinjected with H3T3ph-specific antibody. After ~ 2 hours, cells were subject to immunofluorescence staining for Aurora B and with secondary antibodies against rabbit IgG to reveal microinjected antibody. Line scans of chromosomes are shown in (E). Yellow highlights the centromere. Scale bars, 5 μm unless noted.



and HeLa cells in which Aurora B and MCAK were delocalized (Fig. 2F and fig. S8, D to F). Thus, Aurora B kinase activation and some of its chromosomal functions appear less sensitive to delocalization of the CPC from inner centromeres than localization of MCAK.

We tested whether H3T3ph plays a direct role in CPC binding at inner centromeres. Biotinylated peptides encompassing residues 1 to 21 of histone H3 with various side-chain modifications were used in “pull-down” experiments from mitotic HeLa cell lysates. Survivin bound strongly to H3(1-21) peptides containing H3T3ph but poorly to unmodified H3(1-21), H4(1-21), or peptides containing H3S10ph or H3T11ph (Fig. 3A and fig. S9A). Borealin, which directly binds Survivin (11, 25), also bound preferentially to H3T3ph peptides (Fig. 3A). Enrichment of Aurora B on H3T3ph peptides was detectable, but weaker than that of Survivin or Borealin (fig. S9A), perhaps because not all CPC components are associated in cell lysates (25). Trimethylation of H3 at Lys⁴ adjacent to T3ph (H3T3phK4me3) strongly diminished association of CPC components with H3T3ph peptides (Fig. 3A and fig. S9A). Two different recombinant forms of the CPC [the full complex, and a subcomplex

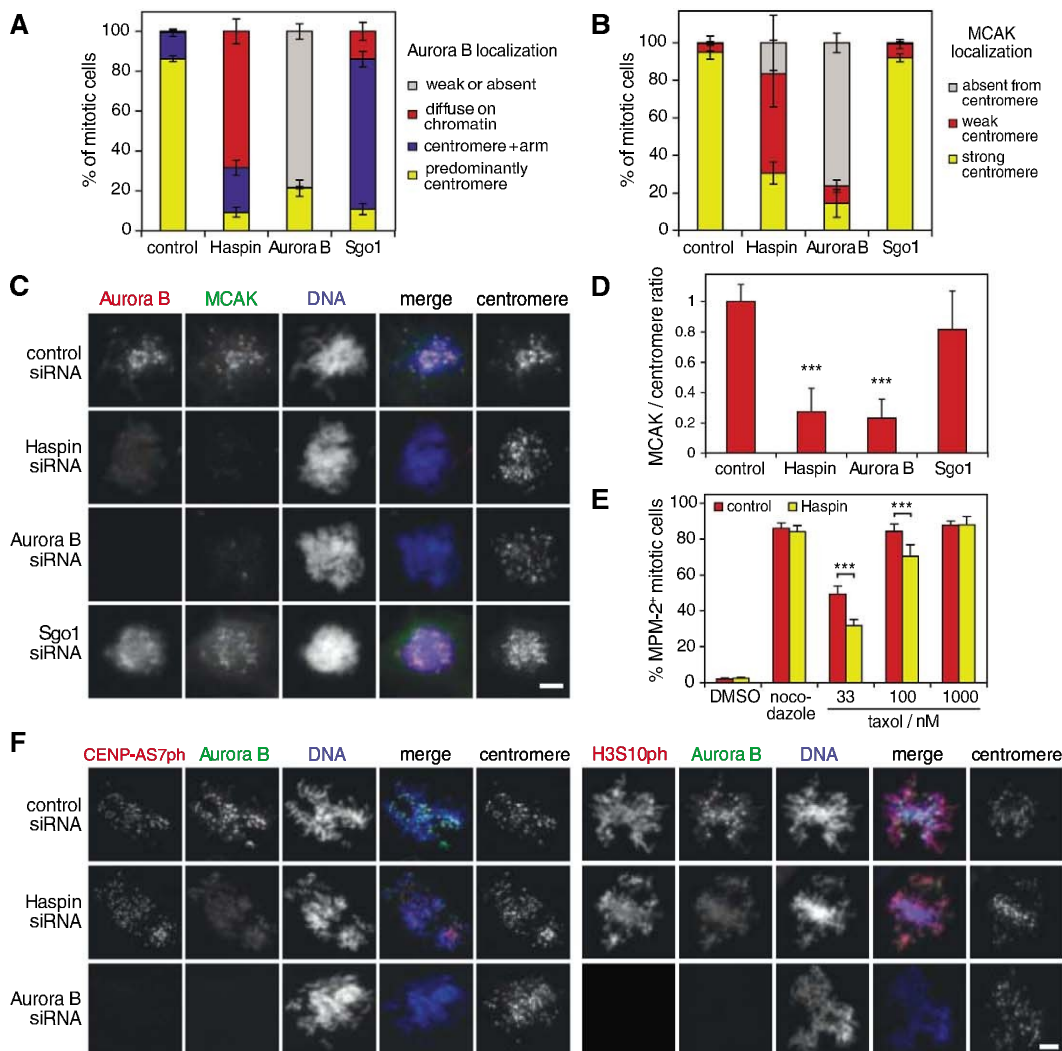
containing INCENP(1-58), Survivin, and Dasra A, a *Xenopus* homolog of Borealin (xISD)] also bound preferentially to H3T3ph peptides, which confirmed that H3T3ph enhances direct binding of the CPC to H3 almost 20-fold (Fig. 3, B and C).

Purified recombinant human Survivin also preferentially bound H3T3ph peptides (Fig. 3D, fig. S9B), consistent with previous reports that Survivin is a key factor targeting the CPC to centromeres (9, 19, 20, 26). Similar to previous observations (20, 27), in HeLa cells depleted of endogenous Survivin, the mutation D70A/D71A (in which Asp⁷⁰ and Asp⁷¹ are replaced by Ala) (7) within the BIR domain prevented Survivin from concentrating at centromeres in mitosis, causing it to become diffusely distributed on chromatin (fig. S10, A to C). Survivin-D70A/D71A still localized to the central spindle, however (fig. S10D). Recombinant Survivin-D70A/D71A showed no binding to H3 peptides in vitro (Fig. 3D), which suggested that the failure of Survivin-D70A/D71A to concentrate at centromeres is due to a failure to recognize H3T3ph.

We then tested whether Survivin-D70A/D71A mimicked the effects of Haspin depletion on CPC

functions. Indeed, Survivin-D70A/D71A supported CPC formation (fig. S11A) and substantial Aurora B autophosphorylation, H3S10 phosphorylation, and CENP-AS7 phosphorylation but did not restore normal localization of Aurora B or MCAK to inner centromeres (Fig. 4, A to C, and fig. S11, B and C) or support spindle checkpoint arrest in the presence of low doses of taxol (fig. S12A). Survivin-D70A/D71A rescued cytokinesis failure caused by Survivin depletion (fig. S12B), but it did not allow normal correction of improper spindle-kinetochore attachments, and an increase in chromosome alignment defects was evident compared with that in cells expressing wild-type Survivin (fig. S12, C and D). Similarly, in chicken DT40 cells expressing Survivin-D72A/D73A (equivalent to D70A/D71A in human Survivin) and in human cells containing Survivin mutated at other sites in the BIR domain, the CPC failed to localize to centromeres, and defects in the spindle checkpoint response to taxol were reported, yet mitotic progression and cytokinesis could still occur (19, 20). Finally, microinjection of H3T3ph-specific antibody into LLC-PK or *Xenopus* S3 cells also caused mitotic defects consistent with loss of Aurora B activity at centromeres, while leaving

Fig. 2. Haspin RNAi delocalizes centromeric MCAK and compromises the spindle checkpoint response but has minor effects on Aurora B activity toward CENP-AS7 and H3S10. (**A** and **B**) U2OS cells were transfected with siRNA, treated with nocodazole for 1 to 2 hours, and subjected to immunofluorescence staining. In each condition, ~100 mitotic cells were classified according to the localization of Aurora B (**A**) or MCAK (**B**). Means $\pm$ SD are shown ($n = 3$). (**C**) Example images of cells treated as above. (**D**) Following treatment as above, the MCAK/centromere autoantigen intensity ratio was determined at ~30 centromeres in 10 or 11 mitotic cells per condition. Means $\pm$ SD are shown ($n = 3$). (**E**) HeLa cells were transfected with siRNA, treated with thymidine for 24 hours, and released into medium containing the compounds indicated. After 18 hours, mitotic indices were determined by flow cytometry with MPM-2 antibody. Means $\pm$ SD are shown ($n = 4$). In (**D**) and (**E**), *** $P < 0.001$ by Bonferroni's multiple comparison test compared with corresponding control. (**F**) Immunofluorescence microscopy of CENP-AS7ph and H3S10ph in mitotic siRNA-transfected U2OS cells. Scale bars, 5 μ m.



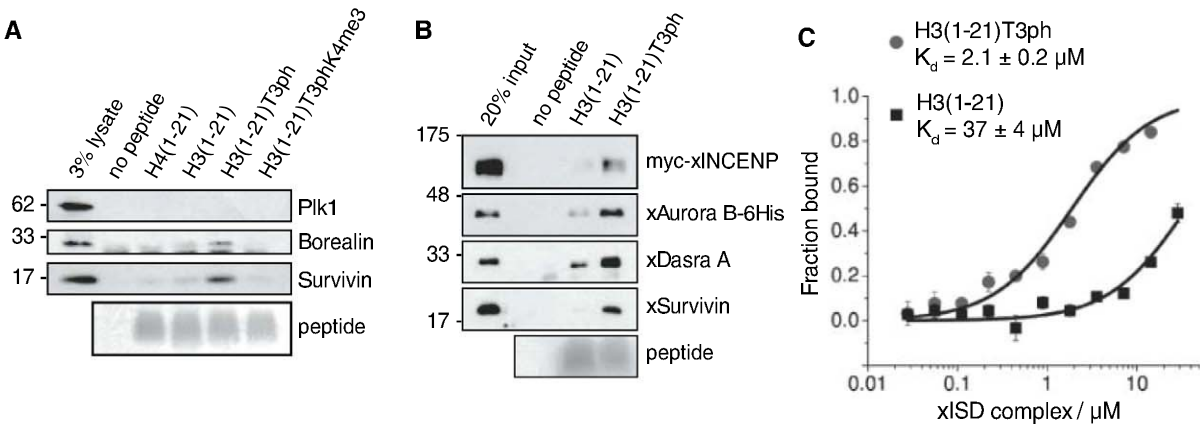


Fig. 3. The CPC binds H3T3ph through Survivin. **(A)** Lysates of nocodazole-arrested HeLa cells were incubated with bead-immobilized histone peptides. Protein binding was visualized by immunoblotting, and peptide loading by Coomassie blue staining. **(B)** Binding of recombinant *Xenopus* CPC to H3 peptides analyzed as above. **(C)** Binding of *Xenopus* ISD complex to H3 peptides measured by fluorescence anisotropy. Means $\pm$ SD are shown ($n = 3$). **(D)** Binding of recombinant human His₈-tagged Survivin to H3 peptides visualized by Coomassie blue staining.

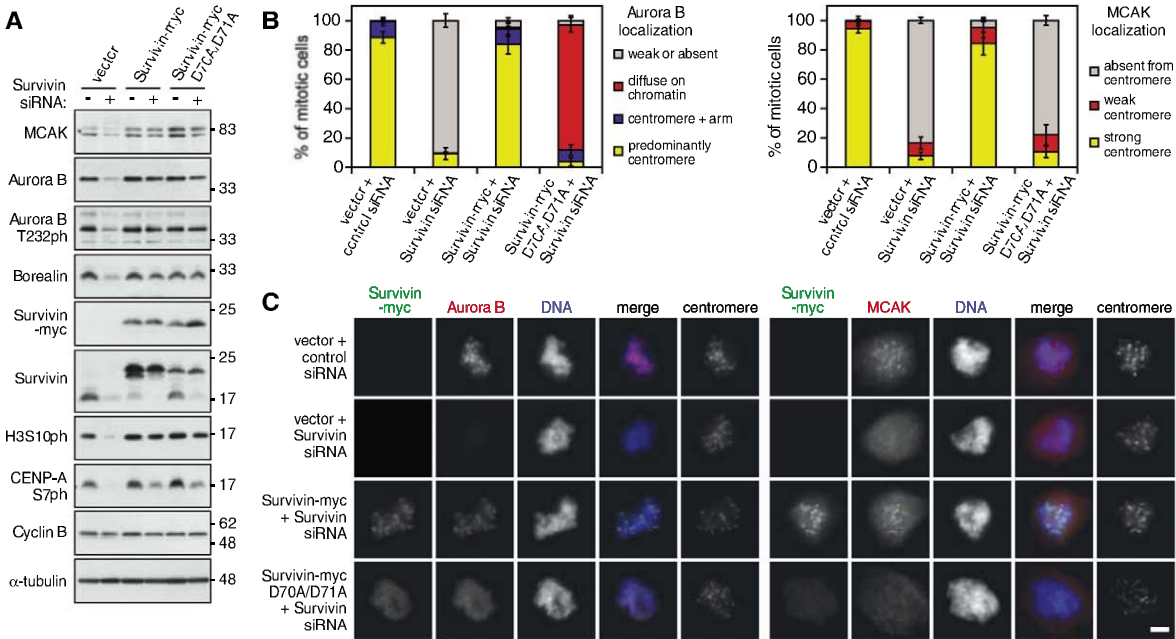


Fig. 4. CPC function at centromeres requires Survivin binding to H3T3ph. **(A)** Lysate immunoblotting of nocodazole-arrested HeLa cells stably expressing Survivin-myc (wild-type or D70A/D71A mutant) and transfected with siRNA as indicated. **(B)** The localization of Aurora B and MCAK was classified by immunofluorescence microscopy in $\sim$ 100 HeLa cells per condition. Means $\pm$ SD are shown ($n = 3$). **(C)** Example images of cells treated as in **(B)**. **(D)** A *Xenopus* S3 cell microinjected with H3T3ph-specific antibody exits mitosis in the presence of unaligned chromosomes (yellow arrows) with clear formation of a cleavage furrow (red arrows). Time stated in minutes:seconds. Three of three injected S3 cells behaved similarly. See movie S2. Scale bars, 5 μ m.

intact its function in cytokinesis (Fig. 4D and movies S1 to S4), which further supported a role for H3T3ph in centromeric CPC function.

Our results are consistent with a model in which H3T3ph generated by Haspin enhances the binding of the CPC to centromeric chromatin and suggest a molecular basis for the importance of the Survivin BIR domain (9, 19, 20, 27). BIR domains in other molecules use the equivalent of residue Asp⁷¹ (D71) to bind the N-terminal Ala of the N-terminal “inhibitor of apoptosis (IAP)–binding motif” (IBM) of proteins such as Smac/DIABLO (28). Survivin binds Smac/DIABLO weakly, but the relevance of this interaction is unclear (29, 30). Instead, the IBM-binding region of the Survivin BIR domain may recognize the N-terminal Ala-Arg-Thr-Lys sequence of histone H3 when Thr³ is phosphorylated, which raises the possibility that other BIR domains act as phospho-specific protein recognition modules.

H3T3ph does not appear to be required for chromatin association or activity of the CPC per se. Rather, H3T3ph contributes to accurate positioning of the CPC at inner centromeres. In this respect, the CPC may resemble heterochromatin protein HP1, which binds histone H3 methylated at Lys⁹ but can also interact with chromatin by Lys⁹ methylation-independent mechanisms (31). In addition, H3T3ph prevents H3 from competitively inhibiting Aurora B autophosphorylation, which could facilitate Aurora B activation at inner centromeres (32). Therefore, Haspin might act through H3T3ph to both position and modulate activation of Aurora B at centromeres.

Loss of inner centromeric CPC accumulation has similar differential effects on centromeric Aurora B functions whether caused by Haspin

depletion or Survivin-D70A/D71A mutation. Our results imply that proximity to inner centromeric Aurora B is not the sole determinant of substrate phosphorylation at centromeres, likely because substrates have different susceptibilities to phosphorylation and dephosphorylation by Aurora B and phosphatases (fig. S1). It may be valuable to further test the idea that Aurora B target sites are “programmed” to respond differently to microtubule-dependent displacement from the inner centromere, which would allow sophisticated regulation of kinetochore and centromere functions in response to tension.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1189435/DC1
Materials and Methods

Figs. S1 to S12

References

Movies S1 to S4

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Survivin Reads Phosphorylated Histone H3 Threonine 3 to Activate the Mitotic Kinase Aurora B

Alexander E. Kelly,^{1,*†} Cristina Ghenoiu,^{1,2,*} John Z. Xue,¹ Christian Zierhut,¹ Hiroshi Kimura,³ Hironori Funabiki^{1†}

A hallmark of mitosis is the appearance of high levels of histone phosphorylation, yet the roles of these modifications remain largely unknown. Here, we demonstrate that histone H3 phosphorylated at threonine 3 is directly recognized by an evolutionarily conserved binding pocket in the BIR domain of Survivin, which is a member of the chromosomal passenger complex (CPC). This binding mediates recruitment of the CPC to chromosomes and the resulting activation of its kinase subunit Aurora B. Consistently, modulation of the kinase activity of Haspin, which phosphorylates H3T3, leads to defects in the Aurora B–dependent processes of spindle assembly and inhibition of nuclear reformation. These findings establish a direct cellular role for mitotic histone H3T3 phosphorylation, which is read and translated by the CPC to ensure accurate cell division.

The accurate segregation of chromosomes during mitosis depends on a complex signaling network, whose spatiotemporal coordination is achieved in part by restricting the activity of kinases to distinct subcellular

structures (1). The kinase Aurora B, together with INCENP, Dasra (also known as Borealin), and Survivin, form the chromosomal passenger complex (CPC), which plays multiple roles during mitosis and meiosis by changing its localization (2).

At the beginning of M phase, the CPC is localized to chromosomes, where it controls chromatin-dependent spindle assembly (3, 4) and processes at the centromere (2). During anaphase, the complex dissociates from chromosomes and relocates to the spindle midzone to stimulate cytokinesis (2). The mechanism by which the CPC is recruited to chromosomes in a cell cycle-specific manner remains unknown.

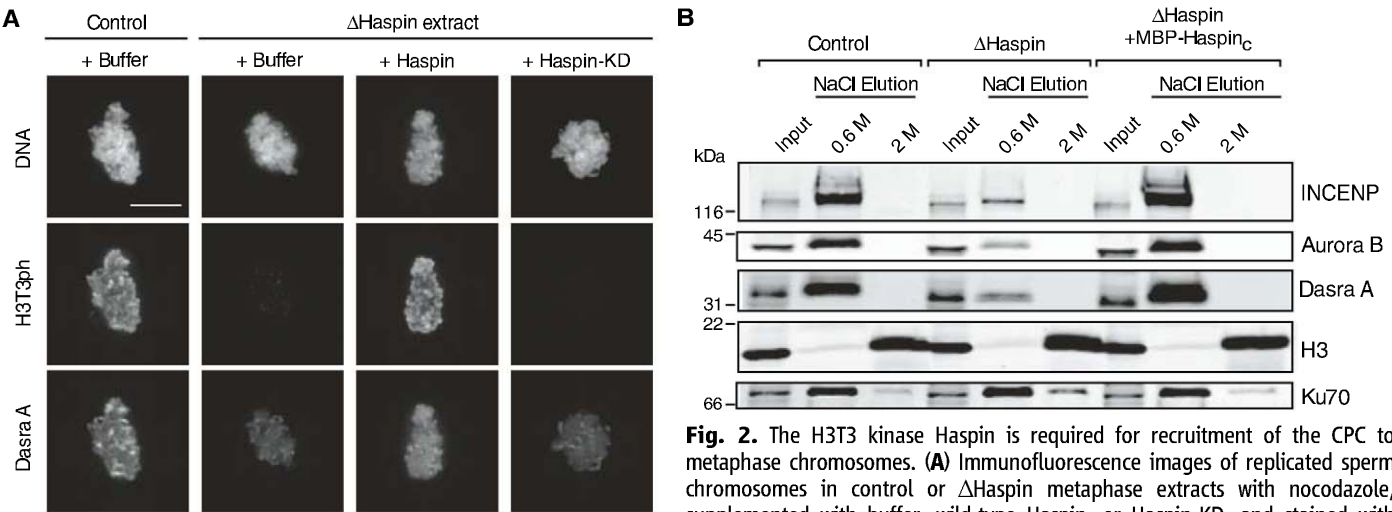
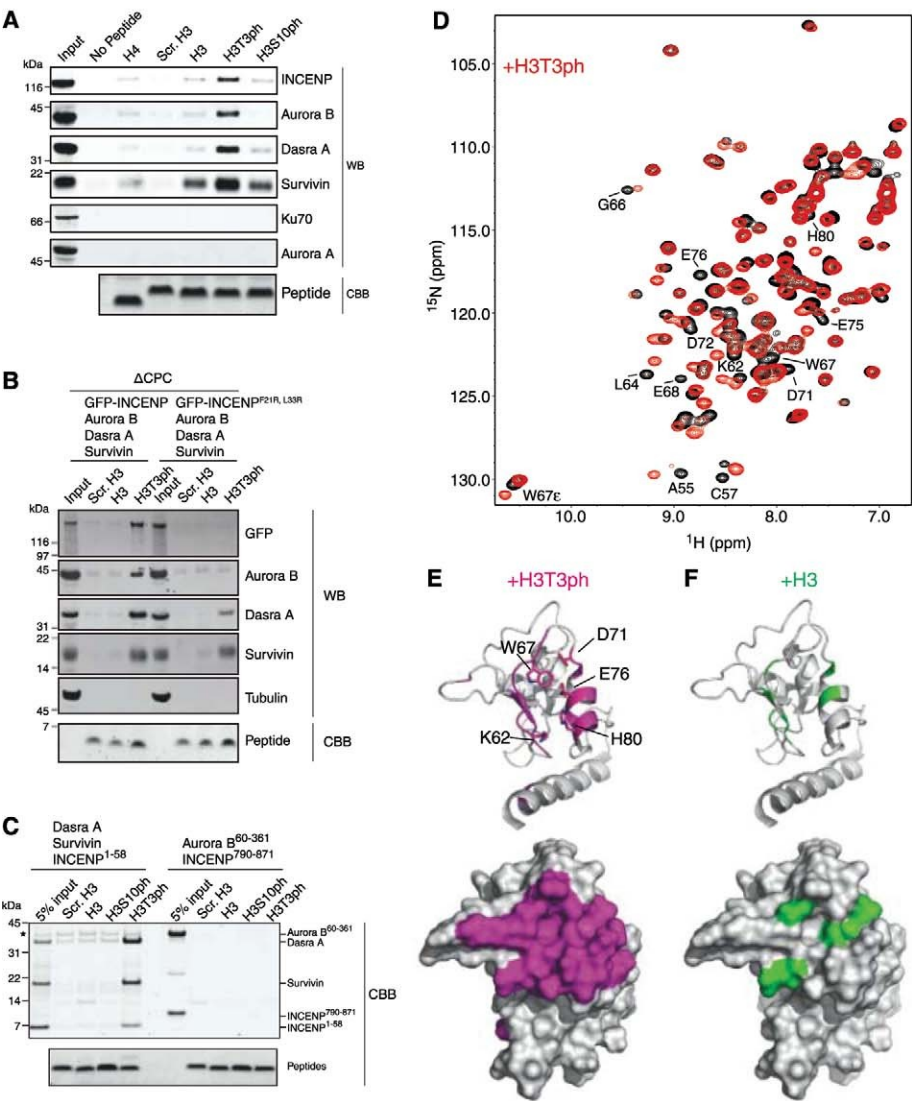
In screening for mitotic histone-binding proteins, we found that the CPC interacts with histones H3/H4 in *Xenopus* cytosolic factor (CSF)-arrested egg extracts (fig. S1) (5). Given that Ser10 of histone H3 (H3S10) is an excellent substrate of Aurora B, we surmised that the CPC–H3/H4 interaction was mediated by the H3 tail (6, 7). In egg extracts, the CPC did not show appreciable bind-

¹Laboratory of Chromosome and Cell Biology, The Rockefeller University, New York, NY 10065, USA. ²Weill Cornell Graduate School of Biomedical Sciences, Department of Molecular Biology, Cornell Medical School, New York, NY 10021, USA. ³School of Frontier Biosciences, Osaka University, Osaka 565-0871, Japan.

*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: funabiki@rockefeller.edu (H.F.); akelly@rockefeller.edu (A.E.K.)

Fig. 1. Survivin mediates the interaction between the CPC and H3T3ph. **(A)** Western blots (WB) of proteins copurified with indicated peptides from metaphase *Xenopus* egg extracts. Peptides were visualized by use of Coomassie brilliant blue (CBB). **(B)** Proteins copurified with indicated peptides from Δ CPC extracts supplemented with recombinant Aurora B and the indicated mRNAs. **(C)** Purified ternary complex consisting of INCENP₁₋₅₈-His₆, Dasra A, and Survivin or the kinase complex consisting of Aurora B₆₀₋₃₆₁ and INCENP₇₉₀₋₈₇₁ was incubated with the indicated peptide beads. Coomassie staining of input and bead fractions is shown. Asterisk indicates contaminating *E. coli* protein. **(D)** Superposition of ¹H, ¹⁵N-HSQC spectra (red) with and (black) without addition of 0.4 mM H3T3ph peptide (1 to 13) to a 0.2 mM ¹⁵N-labeled human Survivin (1 to 120). Some of the NMR signals of the free protein that shift upon titration with peptide are shown. **(E)** H3T3ph (1 to 13) binding surface of Survivin (1 to 120). Magenta indicates residues whose resonances substantially shift upon addition of the peptide. **(F)** Unmodified H3 (1 to 20) binding surface of Survivin (1 to 120). Green indicates residues whose resonances substantially shift upon addition of the peptide.



indicated antibodies. Scale bar, 5 μ m. **(B)** Western blots of proteins copurified with DNA beads from control or Δ Haspin extracts supplemented with buffer or 2.5 μ M MBP-Haspin_c. Proteins were eluted from beads first with 0.6 M NaCl and then with 2M NaCl. Ku70, a DNA-binding protein, serves as a loading control.

Fig. 3. Haspin controls chromatin-induced Aurora B activation to promote spindle assembly. **(A)** Plasmid DNA was incubated for the indicated times at 20°C with control egg extracts, Δ Haspin extracts, or control extracts containing 2.5 μ M MBP-Haspin_c. Aurora B phosphorylation at T248 and hyperphosphorylation of Op18 (arrowhead) were monitored by means of Western blot. **(B)** Representative images of bipolar spindles formed on replicated sperm chromosomes in control or Δ Haspin extracts. Blue, DNA; red, rhodamine-tubulin. Scale Bar, 10 μ m. **(C)** Histogram of metaphase spindle length in control and Δ Haspin extracts as measured in table S1. Error bars represent ranges of three experiments.

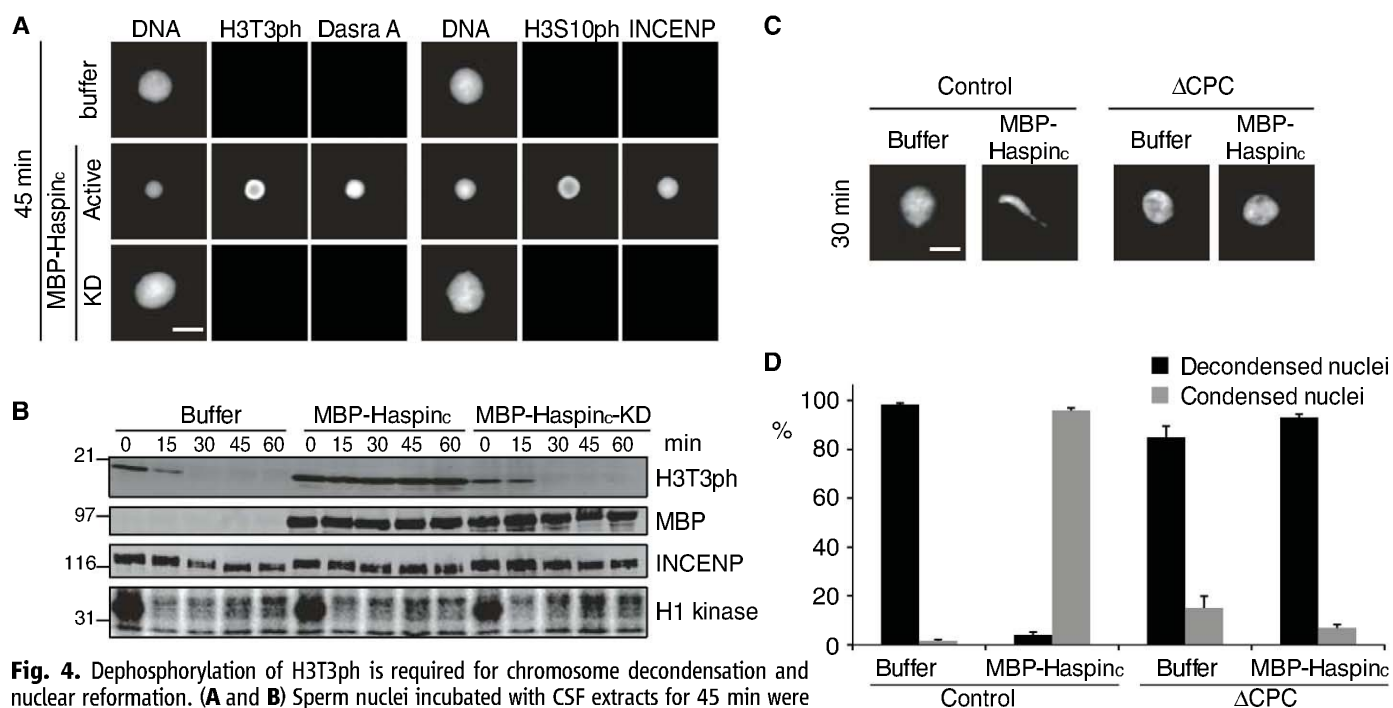
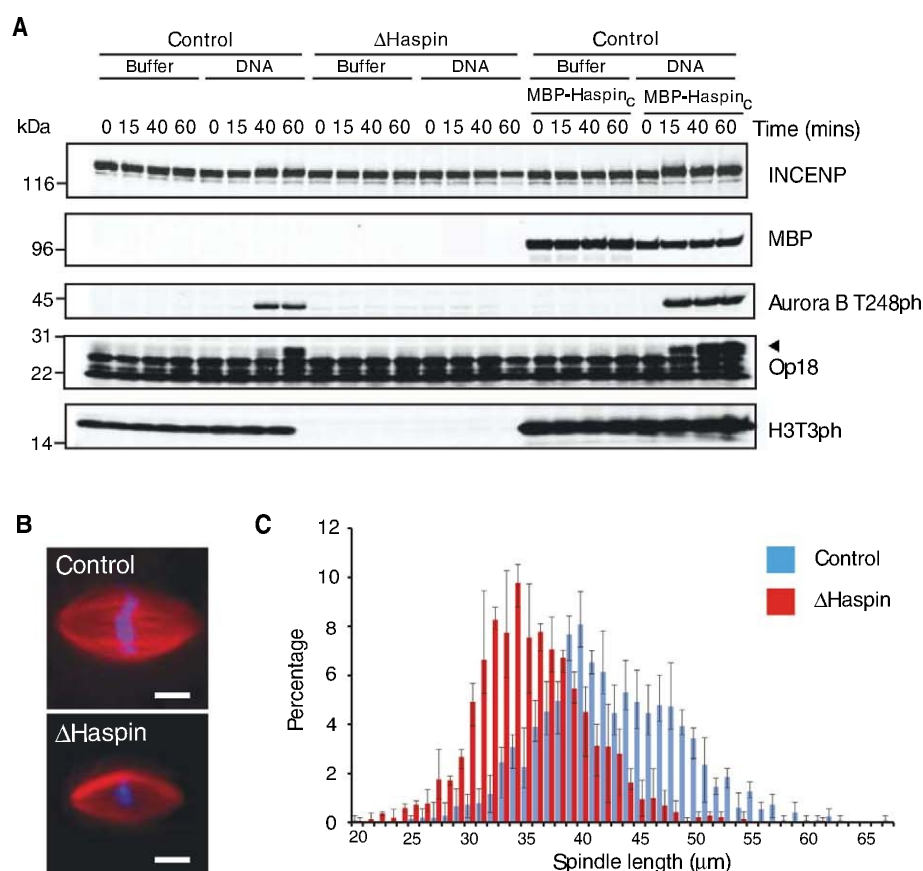


Fig. 4. Dephosphorylation of H3T3ph is required for chromosome decondensation and nuclear reformation. **(A and B)** Sperm nuclei incubated with CSF extracts for 45 min were subsequently treated with buffer, 2.5 μ M MBP-Haspin_c, or MBP-Haspin_c-KD, together with calcium to induce exit from metaphase. **(A)** Indirect immunofluorescence 45 min after calcium addition. **(B)** Western blot analysis and histone H1 kinase assay (autoradiography). **(C and D)** Sperm nuclei and calcium were added together to CSF control or Δ CPC extracts containing buffer or MBP-Haspin_c. **(C)** Hoechst 33258 staining at 30 min after calcium addition. **(D)** Quantification of **(C)**. Mean and SEM of three independent experiments ($n > 30$ sperm nuclei). Scale bars, 10 μ m.

ing to a peptide corresponding to the N-terminal tail of H3 (H3₁₋₂₁) (Fig. 1A). However, all four CPC subunits bound to an H3 peptide phosphorylated on Thr3 (H3T3ph) more strongly than to H3₁₋₂₁, H3S10ph, scrambled H3 sequence peptide (Scr. H3), or a histone H4 N-terminal (H4₁₋₁₈) peptide (Fig. 1A).

The CPC can be separated into two functional modules (fig. S2A): the kinase module consisting of Aurora B and the C-terminal domain of INCENP (IN box) (8, 9) and the chromosome-localization module consisting of the N-terminal region of INCENP, Survivin, and Dasra, which form a ternary subcomplex through a tight helical bundle (4, 10). By introducing two mutations in INCENP (F21R and L33R) that disrupt this helical bundle (10) (fig. S2B), we found that the chromosome-localization module is important for recruitment of the CPC to H3T3ph (Fig. 1B) (11). A purified ternary complex of Survivin, Dasra A, and INCENP₁₋₅₈-His₆, but not the kinase module of the CPC (Aurora B₆₀₋₃₆₁-INCENP₇₉₀₋₈₇₁) (9), specifically bound to H3T3ph peptide *in vitro* (Fig. 1C), demonstrating that the chromosome-localization module of the CPC directly recognizes H3T3ph.

Survivin was able to bind H3T3ph peptides in the absence of other CPC subunits (fig. S2C), implying that it is the receptor for H3T3ph. We further defined this interaction using nuclear magnetic resonance (NMR) spectroscopy, taking advantage of published resonance assignments of human Survivin (12). Analysis of [¹⁵N, ¹H]-heteronuclear single-quantum coherence spectroscopy (HSQC) spectra of Survivin in the presence of increasing amounts of H3T3ph peptide revealed that several resonances were perturbed (Fig. 1D and fig. S3). The migration pattern of these peaks indicated that the interaction was in slow chemical exchange and came to saturation (fig. S3A), confirming a direct and strong interaction. When mapped onto the structure of Survivin (12), the chemical shift perturbations induced by H3T3ph peptide clustered to form a contiguous binding pocket in its BIR domain (Fig. 1E). Addition of unmodified H3 peptide to Survivin induced only a small subset of these chemical shift perturbations (fig. S3, B and C) demonstrating that in the absence of phosphorylation, H3 interacts with Survivin far less extensively (Fig. 1F). Among the residues within the H3T3ph-binding pocket is D71, which is critical for centromeric localization and function of the CPC *in vivo* (13, 14).

The binding interface of Survivin and H3T3ph is highly similar to that of the BIR3 domain of XIAP (X-linked inhibitor of apoptosis) and the N terminus of processed SMAC (15, 16) or caspase-9 (17) (fig. S4). In particular, E314 of XIAP (equivalent to Survivin D71) has been shown to coordinate the N-terminal alanine of its ligands and its mutation abrogates binding *in vitro* (15, 16), suggesting that recognition of the N-terminal alanine of histone H3 by Survivin is carried out in the same manner with D71 (fig. S4D).

In contrast, Survivin basic residues H80 and K62, which are not conserved in the BIR3 domain of XIAP, shift upon H3T3ph binding (figs. S3C and S4) and may accommodate the negatively charged phosphate on H3T3.

To further investigate the physiological importance of the CPC-H3T3ph interaction, we depleted the *Xenopus* homolog of the mitotic H3T3 kinase Haspin (18) from egg extracts (figs. S5 and S6). In control extracts, H3T3ph was found along entire chromosomes, whereas Dasra A and INCENP localized to chromosome arms and were particularly enriched at centromeres (Fig. 2A and fig. S7A). Haspin depletion abolished the majority of H3T3ph and, concomitantly, significantly reduced Dasra A and INCENP signals from chromosomes (Fig. 2A and figs. S7 and S8). The kinase activity of Haspin is important for chromosomal recruitment of the CPC because addition of wild-type Haspin, but not a kinase dead (KD) version (18), to Δ Haspin extracts rescued the ability of Dasra A and INCENP to bind chromosomes (Fig. 2A and figs. S7 and S8).

We next biochemically tested the effect of Haspin depletion on binding of the CPC to chromatin. In egg extracts, DNA beads assemble functional chromatin, recruiting the CPC and supporting bipolar spindle formation (3, 4). Consistent with Haspin's role in localizing the CPC to chromosomes, binding of the CPC to DNA-beads was reduced in Δ Haspin extracts, and this defect was rescued by recombinant C-terminal kinase domain of Haspin (MBP-Haspin_C) (Fig. 2B and fig. S9). However, Haspin does not appear to serve as a physical bridge to mediate the CPC-chromatin interaction because the CPC bound H3T3ph peptides in Δ Haspin extracts (fig. S10).

Chromatin assembled on DNA stimulated Aurora B autophosphorylation of its activation loop at threonine 248 (T248ph) (9) and hyperphosphorylation of the Aurora B substrate Op18 (4) in metaphase egg extracts (Fig. 3A). Chromatin failed to stimulate Aurora B activity in Δ Haspin extracts, whereas excess MBP-Haspin_C accelerated the kinetics of chromatin-dependent Aurora B phosphorylation. Furthermore, MBP-Haspin_C rescued the inability of DNA and sperm chromosomes to activate Aurora B in Δ Haspin extracts (fig. S11), establishing the role of Haspin in chromatin-dependent activation of Aurora B. However, it is unlikely that Haspin directly controls intrinsic Aurora B activity because it is not required for activation of Aurora B by stabilized microtubules, which is a chromatin-independent activator (fig. S11) (4, 7).

Chromatin-induced Aurora B activation is required to drive spindle assembly around chromosomes by suppressing microtubule-depolymerizing activities in *Xenopus* egg extracts (3, 4). In Δ Haspin extracts, this function was compromised, resulting in shorter spindle length (Fig. 3, B and C, and table S1). Although spindles can still form, which may be attributable to residual CPC on chromosomes (Fig. 2 and fig. S8), these results suggest that direct recruitment of the CPC by H3T3ph

activates Aurora B on chromosomes to promote spindle assembly.

At the metaphase-to-anaphase transition, the CPC dissociates from chromosomes (2), which is a process critical to subsequent chromosome decondensation and nuclear reformation (19). Because H3T3ph is dephosphorylated upon exit from M phase (18, 20, 21) (fig. S6B), we investigated whether its removal is essential to these processes in egg extracts. Metaphase chromosomes, preassembled in CSF control extracts, were decondensed 45 min after calcium addition, which induces transitioning to interphase (Fig. 4A). At this point, Dasra A and INCENP dissociated from chromosomes, H3T3ph and H3S10ph levels decreased (Fig. 4A), and functional nuclear formation was nearly complete (fig. S12, A and B). However, in extracts containing constitutively active MBP-Haspin_C, but not MBP-Haspin_C-KD, chromosomes remained condensed with robust staining of Dasra A, INCENP, H3T3ph, and H3S10ph, and nuclear import was not initiated (Fig. 4A and fig. S12, A and B). These phenotypes were not due to a delay in cell cycle transition because H1 kinase activity was not detectable 15 minutes after calcium addition in all samples (Fig. 4B). The chromosome decondensation defect caused by MBP-Haspin_C was CPC-dependent, as expected if Haspin worked through CPC activation (Fig. 4, C and D, and fig. S12C).

Our study demonstrates that M phase-specific phosphorylation of histone H3 at Thr3 recruits the CPC to chromatin to activate Aurora B and that this interaction is mediated by Survivin (fig. S13). This interaction controls spindle assembly and nuclear reformation in *Xenopus* egg extracts and is important for spindle checkpoint signaling at the centromere in human cells (22). These results highlight a mechanism by which the cell cycle control of Haspin activity restricts chromosomal Aurora B localization and activation to M phase through histone modification.

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11. In the mutants, other amino acids were substituted at certain locations; for example, F21R indicates that phenylalanine at position 21 was replaced by arginine. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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23. The GenBank accession number for *X. laevis* Haspin is HM559585. We thank M. Dasso, J. Higgins, and S. Mochida for reagents; T. Maniar and K. Yap for technical assistance; J. Higgins for sharing results; and the Rockefeller University Bio-Imaging Resource Center. This work was supported by a Charles H. Revson Biomedical Fellowship (A.E.K.), Marie-Josée and Henry Kravis and Erwin Schrödinger postdoctoral fellowships (C.Z.), grants-in-aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (H.K.) and NIH (GM075249, H.F.).

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Materials and Methods

Figs. S1 to S13

Table S1

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Two Histone Marks Establish the Inner Centromere and Chromosome Bi-Orientation

Yuya Yamagishi,^{1,2*} Takashi Honda,^{1,3*} Yuji Tanno,¹ Yoshinori Watanabe^{1,2,3†}

For proper partitioning of chromosomes in mitosis, the chromosomal passenger complex (CPC) including Aurora B and survivin must be localized at the center of paired kinetochores, at the site called the inner centromere. It is largely unknown what defines the inner centromere and how the CPC is targeted to this site. Here, we show that the phosphorylation of histone H3–threonine 3 (H3–pT3) mediated by Haspin cooperates with Bub1-mediated histone 2A–serine 121 (H2A–S121) phosphorylation in targeting the CPC to the inner centromere in fission yeast and human cells. H3–pT3 promotes nucleosome binding of survivin, whereas phosphorylated H2A–S121 facilitates the binding of shugoshin, the centromeric CPC adaptor. Haspin colocalizes with cohesin by associating with Pds5, whereas Bub1 localizes at kinetochores. Thus, the inner centromere is defined by intersection of two histone kinases.

The accurate segregation of chromosomes during cell division requires sister chromatids to be captured by microtubules from the opposite spindle poles (bi-orientation), which is accomplished by a trial-and-error process of kinetochore-microtubule attachment that relies on Aurora B kinase locating at centromeres (1–6). Shugoshin, a conserved inner centromere protein protecting centromeric cohesion (7, 8), also mediates chromosomal passenger complex (CPC) targeting to centromeres (9–12). In fission yeast, meiosis-specific shugoshin Sgo1 protects cohesin, a sister chromatid cohesion molecule (13), whereas another shugoshin, Sgo2, mediates CPC targeting to centromeres, thus dividing the labors (9). These functions are shared by two shugoshins in human cells (12, 14). Although Bub1-mediated histone 2A–serine 121 (H2A–S121) phosphorylation is important for the centromere targeting of shugoshin and the CPC (15), we are still far from understanding the full picture of CPC targeting to the inner centromere. More fundamentally, it is unknown how the chromatin locus of the inner centromere is established.

Haspin is a highly conserved kinase and might function in the centromeric protection of

cohesin in parallel with shugoshin in human cells (16). To examine the functional link of Haspin to shugoshins in fission yeast, we deleted the *hrk1*⁺

gene (SPAC23C4.03) encoding Haspin-related kinase (17). *Hrk1* is dispensable for centromeric cohesion in both mitosis and meiosis (fig. S1, A and B), meaning that *Hrk1* is not relevant to cohesion or Sgo1 function. However, *hrk1Δ* cells show synthetic lethality (or severe sickness) with the mutation of another shugoshin Sgo2, the centromeric CPC adaptor (Fig. 1A) (9, 12). Like *sgo2Δ* cells, *hrk1Δ* cells impair CPC localization to centromeres in metaphase (fig. S1C). The *hrk1Δ sgo2Δ* double mutant nearly entirely abolishes CPC targeting to centromeres accompanied by a high incidence of chromosome lagging or mis-segregation in anaphase (fig. S1, C and D). Notably, *hrk1Δ* does not show a synthetic sickness with *swi6Δ*, a mutation in the heterochromatin protein gene that shows a reduction in centromeric CPC concentration and a synthetic sickness with *sgo2Δ* (Fig. 1A) (9). Thus, *Hrk1* acts in CPC targeting to centromeres in a redundant capacity with Sgo2 but possibly in the same pathway with Swi6.

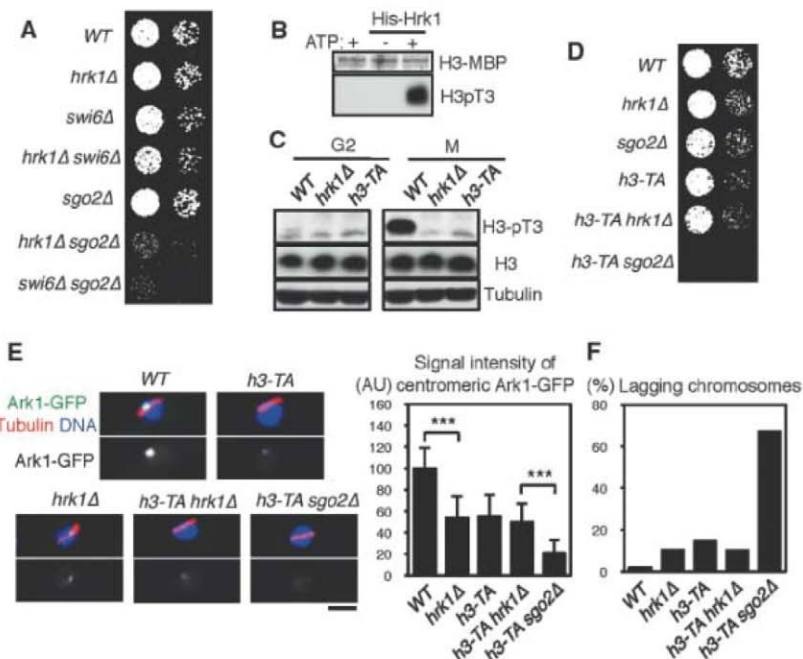


Fig. 1. Phosphorylation of H3-T3 promotes CPC targeting to centromeres. (A) Serial dilution growth assay. **(B)** H3–maltose binding protein (MBP) protein was incubated with His-Hrk1 in the absence or presence of adenosine triphosphate (ATP). H3-T3 phosphorylation was detected by immunoblot. **(C)** Cell extracts prepared from the indicated cells were immunoblotted for H3-pT3 and H3. G2; asynchronous culture, M; prometaphase arrest (by *nda3-KM311*). **(D)** Serial dilution growth assay. **(E)** The signal intensity of Ark1–green fluorescent protein (GFP) in metaphase cells was measured. Error bars represent SD ($n = 20$ cells). Scale bar, 2 μ m. *** $P < 0.001$ (Student's t test). AU, arbitrary units. **(F)** The indicated cells were examined for the frequency of lagging chromosomes at anaphase ($n > 100$ cells).

¹Laboratory of Chromosome Dynamics, Institute of Molecular and Cellular Biosciences, University of Tokyo, Yayoi, Tokyo 113-0032, Japan. ²Graduate Program in Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, Hongo, Tokyo 113-0033, Japan. ³Graduate School of Agricultural and Life Science, University of Tokyo, Yayoi, Tokyo 113-0032, Japan.

*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: ywatanab@iam.u-tokyo.ac.jp

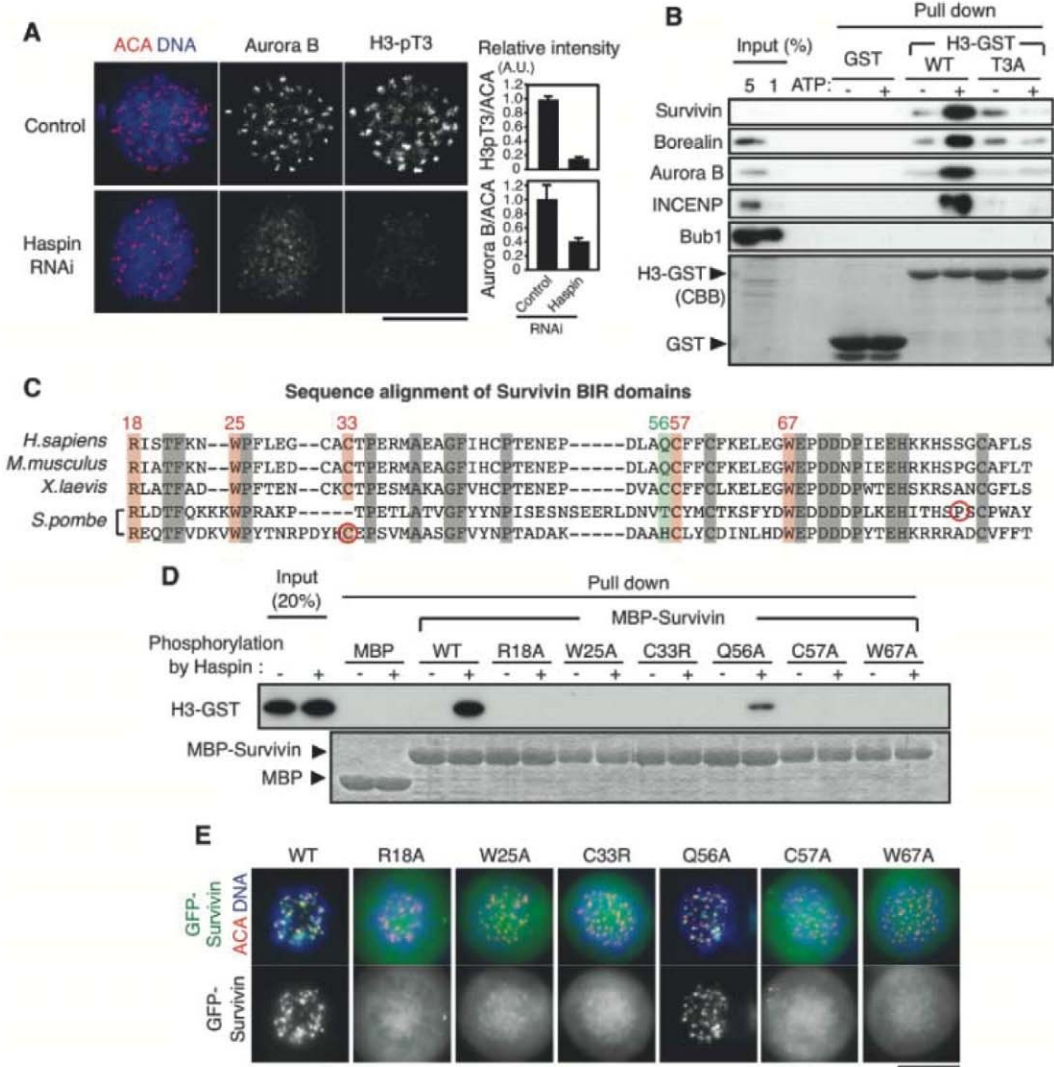
Like human Hapsin (16), fission yeast Hrk1 has the ability to phosphorylate histone H3 at Thr³ (T3) in vitro (Fig. 1B). Immunoblot assays revealed that histone H3-T3 is phosphorylated in prometaphase-arrested cells, but little in asynchronous cells. This phosphorylation is abolished in *hrk1Δ* cells or *h3-1A* cells in which Thr³ is replaced with alanine (H3-TA) in all histone H3 genes (*hht1*⁺, *hht2*⁺, and *hht3*⁺) (Fig. 1C), suggesting that Hrk1 is the sole kinase phosphorylating H3-T3 in vivo. Although *h3-1A* cells are viable as *hrk1Δ* cells, they show a similar level of reduction in centromeric CPC, as well as a defect in chromosome bi-orientation (lagging chromosomes) (Fig. 1, D to F). The *hrk1Δ h3-1A* double mutant shows no cumulative defects, whereas *h3-1A* and *sgo2Δ* show synergetic defects as seen for *hrk1Δ* and *sgo2Δ* (Fig. 1, A and D). These results suggest that Hrk1 acts in CPC targeting to centromeres by phosphorylating H3-T3. Consistently, in vitro binding assays revealed that only when H3-T3 is phosphorylated, histone H3 interacts with the baculoviral IAP repeat (BIR) domain of survivin (fig. S2).

In HeLa cells, depletion of Haspin by treatment with small interfering RNA (siRNA) impaired the centromeric CPC localization, as well as H3-T3 phosphorylation, in mitotic chromosomes (Fig. 2A). A pull-down assay using recombinant H3 revealed that wild-type H3, but not the mutant H3-TA, pulled down the CPC from mitotic extracts only when H3 was phosphorylated by Haspin (Fig. 2B). Point mutations at the conserved amino acids (Arg¹⁸, Trp²⁵, Cys³³, Cys⁵⁷, and Trp⁶⁷) in the BIR domain, but not a mutation at a nonconserved residue (Gln⁵⁶), lost the ability to bind phosphorylated histone H3–threonine 3 (H3-pT3) in vitro (Fig. 2, C and D), whereas they showed intact complex formation with INCENP (inner centromere protein) and borealin (fig. S3). Accordingly, all mutant survivin proteins whose in vitro binding to H3-pT3 was abolished failed to localize at centromeres in vivo (Fig. 2E). These results for human survivin, together with those for fission yeast Bir1 (fig. S2), strongly argue that the conserved BIR domain of survivin plays a crucial role in targeting the CPC to centromeres by binding directly to H3-pT3.

Given that the interaction between Bir1/survivin and H3-pT3 is required for centromere targeting of the CPC, the phosphorylation of H3-T3 might be observed at the inner centromere. Chromatin immunoprecipitation (ChIP) assays in fission yeast indicates that H3-T3 phosphorylation localizes, not only at the pericentromeric region (inner centromere in metazoan cells), but also at the mating-type locus and telomere-adjacent regions in mitosis (Fig. 3A and fig. S4A), whereas Bir1 is enriched mostly at the pericentromeric region (Fig. 3A). Although Hrk1 localizes to all heterochromatic regions like H3-pT3, additional localization appears in the euchromatic regions (Fig. 3A; also see below). As Hrk1 might function in the Swi6 pathway (Fig. 1A), Hrk1 localization was abolished specifically in the heterochromatic regions by *swi6Δ*, whereas it was preserved at the euchromatic regions (Fig. 3A and fig. S4B). Thus, heterochromatin assembly is an important, but not the sole, requirement for chromatin association of Hrk1.

Cohesin is the crucial factor involved in chromosome segregation downstream of hetero-

Fig. 2. The interaction between the BIR domain and H3-pT3 is required for CPC targeting to centromeres in HeLa cells. **(A)** HeLa cells treated with Haspin siRNA were arrested at prometaphase by treatment with nocodazole and MG132 for 3 hours and were immunostained with anti-Aurora B, anti-H3-pT3, and Hoechst 33342. Relative fluorescence intensities of Aurora B and H3-pT3 toward anti-centromere antibodies (ACA) were quantified at 10 centromeres in each cell. Error bars represent SEM (*n* > 10 cells). RNAi, RNA interference. **(B)** Chromatin extracts prepared from mitotic HeLa cells were pulled down by H3–glutathione S-transferase (GST) or GST that had been pre-incubated with His-Haspin in the absence or presence of ATP and were analyzed by immunoblot using the indicated antibodies. **(C)** Alignment of the BIR domain of survivin. *Schizosaccharomyces pombe* survivin has two BIR domains. Mutation sites used in (D) and (E) are marked at the top, and *S. pombe* *bir1-71* mutations are denoted by red circles. **(D)** H3-GST was pre-incubated with His-Haspin and pulled down with MBP-survivin (wild-type and mutant) proteins. **(E)** Mitotic HeLa cells expressing indicated enhanced GFP–survivin were immunostained with ACA and antibodies against GFP. DNA was costained with Hoechst 33342. Scale bars in (A) and (E), 10 μm.



chromatin assembly (18) and might be required for CPC localization (19, 20). We therefore screened cohesin-related proteins for interaction with Hrk1 by the yeast two-hybrid system, revealing that the N terminus of Hrk1 interacts with the cohesin-associating protein Pds5, as well as with Swi6 (Fig. 3B). *pds5Δ* cells lost Hrk1 localization, as well as H3-T3 phosphorylation, along the entire chromosome region (Fig. 3, C and D), whereas Pds5 localization did not depend on Hrk1 (fig. S5A). Consistently, the localization of Hrk1 and Pds5 (and cohesin) coincide at all tested chromosomal sites (Fig. 3D and fig. S5C). By tethering Pds5 at a specific chromosomal site, we observed accumulation of Hrk1 at this site (fig. S6), validating that Pds5 acts as a primary basis for Hrk1 localization. The interaction of Hrk1 with Swi6 might also be important, because Hrk1 localization at the heterochromatic region partly depends on this interaction (fig. S7).

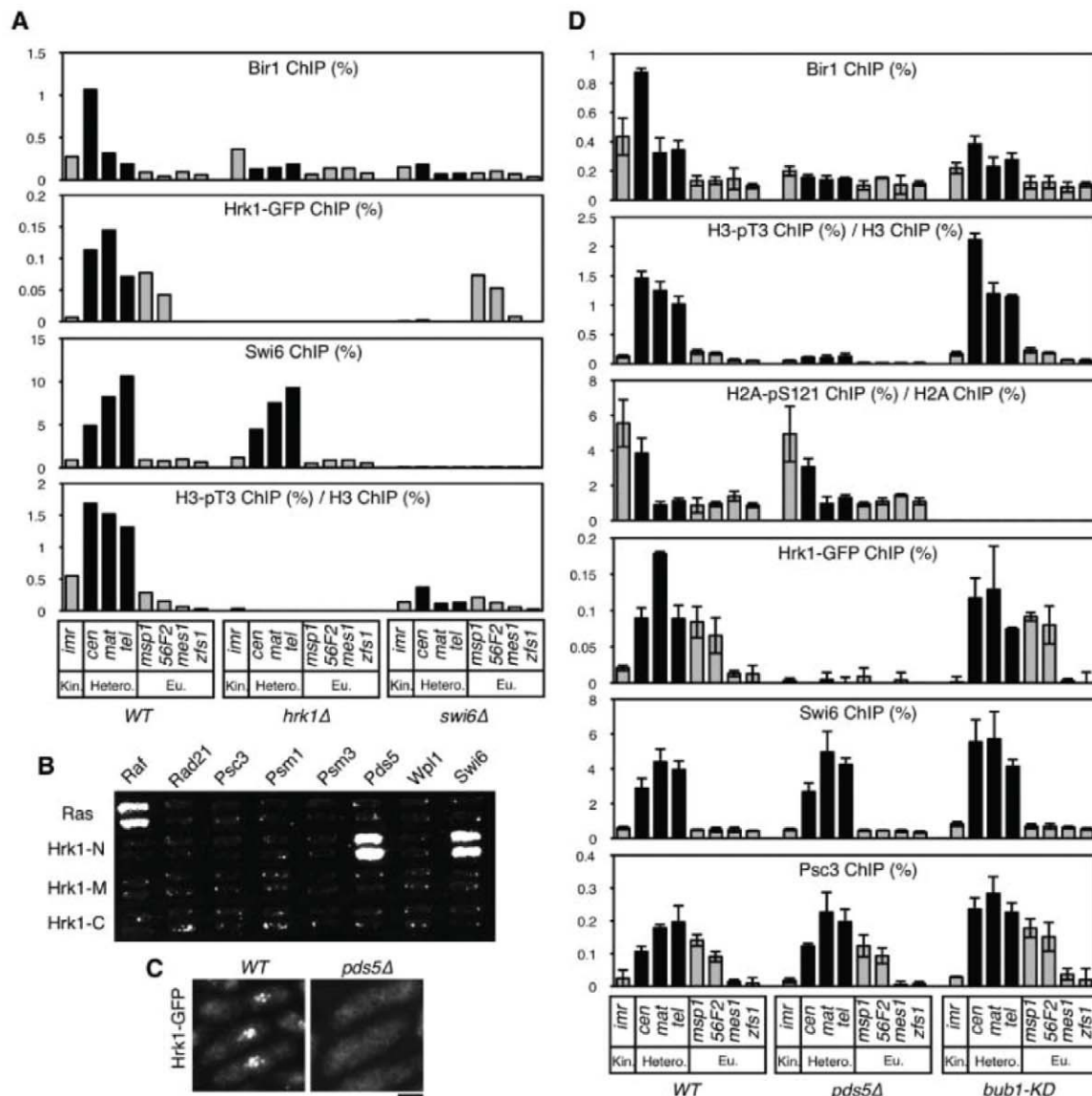
The aforementioned results suggest that the Swi6-Pds5-Hrk1-H3-survivin pathway acts in CPC targeting to centromeres in addition to the pre-

viously identified Bub1-H2A-Sgo2 pathway (15). Genetic analyses of mutations involved in these pathways show that intrapathway double mutations cause no cumulative defects, whereas interpathway double mutations cause synthetic defects in chromosome bi-orientation and cell growth (Fig. 4A). Therefore, the two histone phosphorylations of H3-T3 and H2A-S121 may act in parallel to accomplish targeting the CPC to centromeres. Although either histone mutation *h3-1A* or *h2a-5A* causes moderate defects in CPC targeting to centromeres, the double mutant abolishes CPC localization at centromeres, causing a severe bi-orientation defect (Fig. 4, B and C). If centromere targeting of the CPC is the major function of the phosphorylation of H3-T3 and H2A-S121, then the forced localization of the CPC should restore the defects in the *h3-1A h2a-5A* double mutant or any interpathway double mutant. To test this, we used the Bir1-chromodomain (CD) protein, a fusion of the Bir1 C-terminal end to the CD, which binds to Lys-9-methylated histone H3 principally located at the pericentromeric

heterochromatin (fig. S8) (9). Remarkably, Bir1-CD restored cell viability as well as chromosome bi-orientation of *h3-1A h2a-5A* cells, as it restored viability of all interpathway double mutants tested (Fig. 4, A and C). These results strongly argue that the primary readout of the histone phosphorylation of H3-T3 and H2A-S121 is the localization of the CPC to the inner centromere, a process essential for setting up chromosome bi-orientation. Although H3-pT3 associates with the CPC, H2A-pS121 may associate with shugoshin (15). A recent study has revealed that the Cdk1-dependent phosphorylation of the CPC promotes the binding to shugoshin, and neither protein can fully localize to chromatin without this phosphorylation (12), suggesting that a physical interaction between the CPC and shugoshin creates a synergistic association of these proteins with nucleosomes (Fig. 4D).

Although Bub1 and Hrk1 kinases cooperate in targeting the CPC to centromeres, Bub1 locates at kinetochores (15), whereas Hrk1 locates at cohesin sites along the chromosome (Fig. 3D

Fig. 3. Hrk1 is recruited to cohesin sites through the interaction with Pds5. **(A)** ChIP analysis was used to measure Bir1, Hrk1, Swi6, H3-pT3, and H3 throughout the kinetochore (*imr1*); heterochromatic centromere (*cen*), mating type locus (*mat*), and telomere (*tel*); and euchromatic arm region (*mps1*, *56F2*, *mes1*, and *zfs1*) in the indicated strains arrested at prometaphase (by *nda3-KM311*). Average of two polymerase chain reaction (PCR) amplifications. *WT*, wild type. **(B)** Yeast two-hybrid assay with Hrk1 fragments. The Ras and Raf pair acts as a positive control. **(C)** Hrk1-GFP was detected in the indicated cells arrested at prometaphase. Scale bar, 2 μ m. **(D)** ChIP analysis was used to measure Bir1, H3-pT3, H3, H2A-pS121, H2A Hrk1, Swi6, and Psc3 in the indicated strains arrested at prometaphase. Error bars represent SD ($n = 3$ PCR amplifications). Note that *msp1* and *56F2* correspond to the cohesin peaks, whereas *mes1* and *zfs1* do not.



and fig. S5C). H2A-S121 is phosphorylated, not only in the kinetochore region, but also in the adjacent pericentromeric region, whereas the phos-

phorylation of H3-T3 is distributed to all heterochromatic regions (Fig. 3D). Crucially, Bir1 is enriched mostly at the pericentromeric region,

the intersection of H3-pT3 and H2A-pS121 distributions, and this enrichment is abolished by either *hrk1Δ* or *bub1-KD* (a kinase-dead mutant)

Fig. 4. H3-pT3 and H2A-pS121 act synergistically in CPC targeting to the inner centromere. **(A)** Summary of genetic analyses. **(B)** The signals of Ark1-GFP expressed from the endogenous promoter were measured in metaphase in the indicated cells. Error bars represent SD ($n = 20$ cells). *** $P < 0.001$ (Student's t test). Scale bar, 2 μ m. **(C)** Serial dilution assay. Frequencies of lagging chromosomes were measured in the indicated cells at anaphase ($n > 100$ cells). **(D)** Schematic depiction of the pathways that regulate CPC targeting to centromeres.

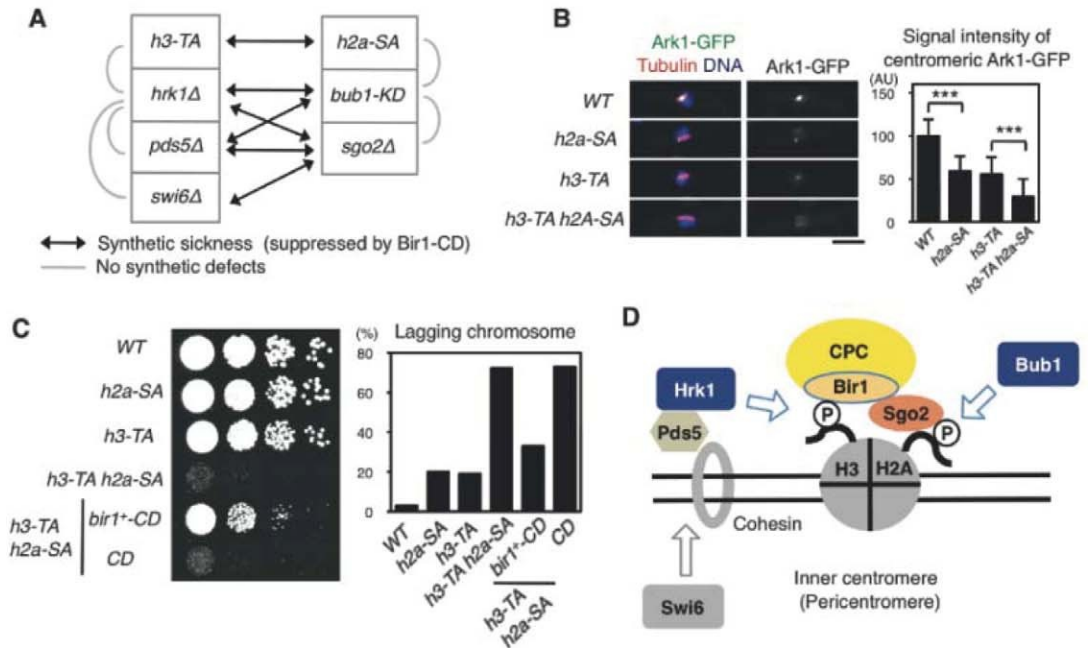
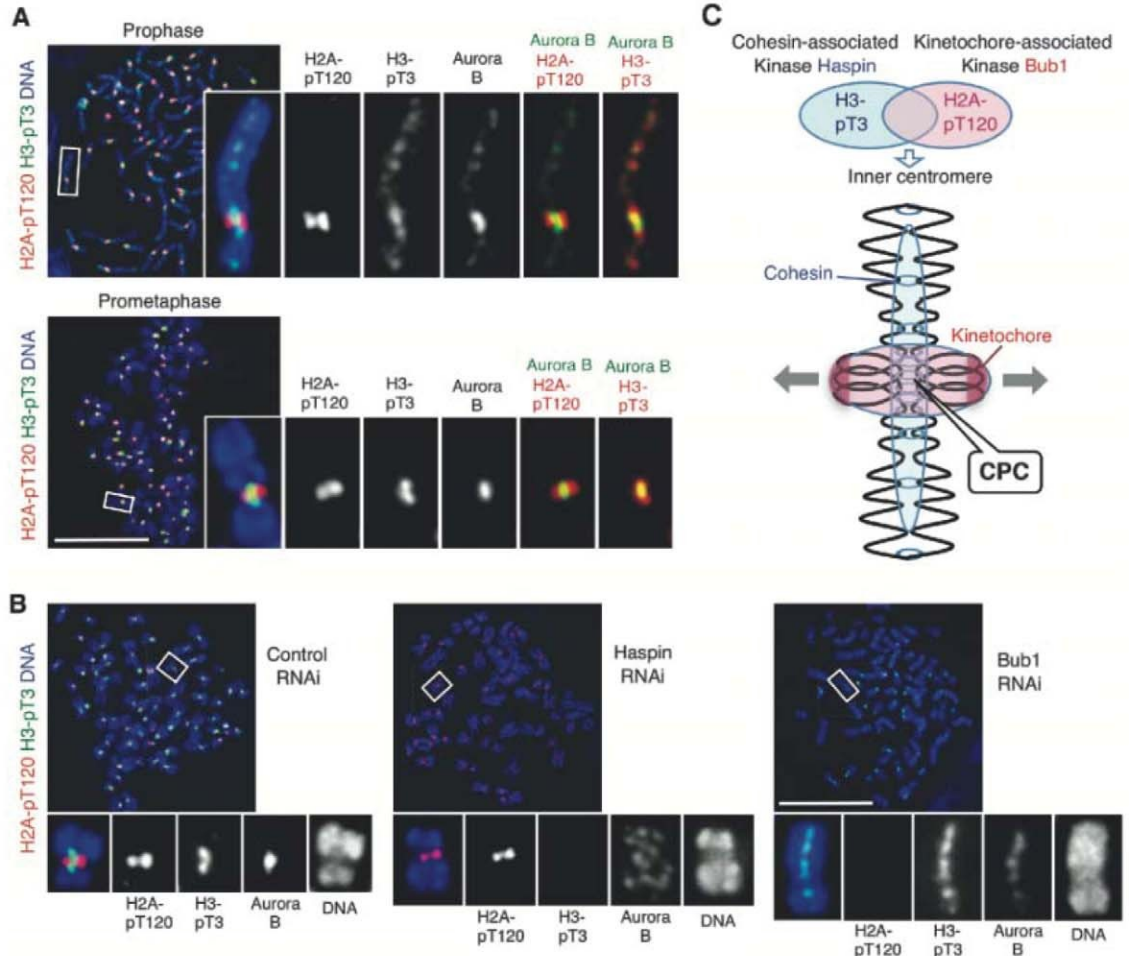


Fig. 5. Aurora B locates at the intersection of H2A-pT120 and H3-pT3. **(A)** Chromosome spreads were prepared from HeLa cells and stained with antibodies against H3-pT3, H2A-pT120, and Aurora B. DNA was costained with Hoechst 33342. Prophase and prometaphase chromosomes are shown (see fig. S9B). Magnified images of paired sister chromatids are shown (insets). **(B)** HeLa cells treated with Haspin or Bub1 siRNAs were arrested at prometaphase by treatment with nocodazole and MG-132 and were examined in chromosome spreads by staining with antibodies against H3-pT3 and H2A-pT120 and Aurora B. More than 90% of spreads in each sample showed the same staining pattern. Magnified images of paired sister chromatids are shown (insets). Scale bars in (A) and (B), 10 μ m. **(C)** Schematic depiction of the definition of the inner centromere.



(Fig. 3, A and D). Residual localization of Bir1 is detected at the noncentromeric heterochromatin regions, which is preserved in *bub1-KD* cells (Fig. 3D). These results imply that the single histone mark H3-pT3 has a moderate ability to recruit the CPC, which otherwise is enriched in the pericentromeric region by the synergetic action of H2A-pS121.

Immunostaining of HeLa cells with antibodies against H2A-pT120 (equivalent to yeast H2A-pS121), H3-pT3, and Aurora B indicates that the H2A-pT120 signals increase as Bub1 accumulates at kinetochores during prophase, and, concomitantly, the distributions of H3-pT3 and Aurora B narrow into regions adjacent to H2A-pT120 (fig. S9A). A zoomed-in view of prophase and prometaphase chromosomes reveals that H3-pT3 signals localize to the intersister region, whereas H2A-pT120 signals locate along the interkinetochore axis, with signals culminating near the kinetochores (Fig. 5A and figs. S9A and S10). Importantly, Aurora B signals are enriched in the merged region of these signals (Fig. 5A), and this Aurora B localization is disrupted by depleting either phosphorylation of histone by RNA interference (Fig. 5B and fig. S11). These results in HeLa cells, together with those in fission yeast, advocate the notion that the inner centromere in eukaryotes is principally defined by the intersection of two histone marks, H2A-pS121 and H3-pT3, that are mediated by cohesin-associated kinase Haspin/Hrk1 and kinetochore-associated kinase Bub1 (Fig. 5C and supporting online material text).

In HeLa cells, the prophase pathway causes a dynamic narrowing of cohesin localization to the centromere (13, 21). For this redistribution, Bub1

kinase plays an important role by localizing H2A-pT120 and shugoshin to the centromere (15, 22), because human shugoshins play a crucial role in cohesin protection (14, 22–24) in addition to binding to the CPC (12). Because Haspin or H3-pT3 localization may also depend on cohesin in HeLa cells (fig. S12), this redistribution of cohesin to centromeres will further attract the CPC-shugoshin, thus constructing a positive feedback loop. Localization interdependencies are observed, not only between the CPC and shugoshin, but also between cohesin and shugoshin in several organisms (9, 16, 21, 25–28). Thus, our study uncovers a fundamental molecular network centered on shugoshin-cohesin-CPC, which couples sister chromatid cohesion to chromosome bi-orientation in eukaryotes.

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Supporting Online Material

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Materials and Methods

SOM Text

Figs. S1 to S12

Table S1

References

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Species Interactions in a Parasite Community Drive Infection Risk in a Wildlife Population

Sandra Telfer,^{1,2*} Xavier Lambin,² Richard Birtles,³ Pablo Beldomenico,⁴ Sarah Burthe,⁵ Steve Paterson,¹ Mike Begon¹

Most hosts, including humans, are simultaneously or sequentially infected with several parasites. A key question is whether patterns of coinfection arise because infection by one parasite species affects susceptibility to others or because of inherent differences between hosts. We used time-series data from individual hosts in natural populations to analyze patterns of infection risk for a microparasite community, detecting large positive and negative effects of other infections. Patterns remain once variations in host susceptibility and exposure are accounted for. Indeed, effects are typically of greater magnitude, and explain more variation in infection risk, than the effects associated with host and environmental factors more commonly considered in disease studies. We highlight the danger of mistaken inference when considering parasite species in isolation rather than parasite communities.

Macroparasites (helminths and arthropods) and microparasites (viruses, bacteria, and protozoa) are integral components of the ecological communities that include their hosts (1), and it is likely that most hosts, most of the time, are infected with more than one parasite species

(2). Interactions between parasites in natural populations, however, have been studied only rarely. A community ecology perspective is particularly relevant for studies of coinfection, as parasites may interact directly by competing for resources or indirectly via the host immune system (3). Inter-

actions may be antagonistic to at least one of the parasites, either as a result of resource shortage or where there are cross-effective immune responses, or they may be beneficial to one or both parasites, as a result of parasite-induced immunosuppression or down-regulation of all or part of the immune system (1).

Experiments in laboratory model systems have demonstrated effects of coinfection on host susceptibility, infection length, and intensity and clinical symptoms (3–5), whereas studies in wildlife populations and humans have established firmly that positive and negative associations can occur between parasites (6–8). Such associations may reflect interactions between parasites. Indeed, interactions between parasites can have substantial effects. For example, human immunodeficiency

¹School of Biological Sciences, University of Liverpool, Crown Street, Liverpool L69 7ZB, UK. ²School of Biological Sciences, University of Aberdeen, Tillydrone Avenue, Aberdeen AB24 2TZ, UK. ³School of Veterinary Science, University of Liverpool, Leahurst Campus CH64 7TE, UK. ⁴Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, RP Kreder 2805, 3080 Esperanza, Santa Fe, Argentina. ⁵Centre for Ecology and Hydrology Edinburgh, Bush Estate, Pentlands, Edinburgh EH26 0QB, UK.

*To whom correspondence should be addressed. E-mail: s.telfer@abd.ac.uk

virus infections are thought to be driving resurgent tuberculosis epidemics in Africa (9). Hence, single parasite studies may yield incorrect or incomplete conclusions. Nonetheless, most epidemiological studies, in animals and humans, still focus on single species. Studies that do consider multiple infections are typically cross-sectional, with each host providing infection data at one time point. The time of initial infection is unknown in such studies. There is, therefore, limited scope for determining whether patterns reflect inherent differences between hosts in either susceptibility or exposure to infection, rather than interactions (10), or for exploring the impact of infection sequence (11). Consequently, in natural populations, the relative importance of interspecific interactions, compared with other factors, in determining the dynamics and structure of parasite communities remains underexplored. Studies that monitor infection status through time are required.

Here, we report an analysis of infection risk using unprecedented time-series data on individual infection histories from four replicate natural populations of field voles, *Microtus agrestis*, comprising 14,075 captures of 5981 individual voles. Populations were sampled every four weeks (May to November 2001; March to November 2002 to 2006), with animals tagged individually and blood samples taken to allow infection status to be monitored over time (12). We investigated individual infection risks for a community of microparasites consisting of cowpox virus, *Babesia microti*, *Bartonella* spp. and *Anaplasma phagocytophilum* (see Table 1 for details of their biology). Infection risk will depend on both the probability of encountering an infectious dose and the probability of infection given exposure (host susceptibility). We aimed to determine whether susceptibility to infection by a microparasite was influenced by other microparasites. Therefore, for each microparasite, we investigated whether the other microparasites influenced the probability that a susceptible animal became infected at a given time point (t_0), adding infection status for these other microparasites as explanatory variables to baseline statistical models that accounted for environmental and individual variables (e.g., sex and season) (12) (table S1). Thus, we guard against detecting spurious associations, which, in reality, reflect correlated exposure risk (e.g., a positive association simply because both parasites are most prevalent in late summer). For parasites causing self-limiting infections, infection status at both t_0 and/or the previous month (t_{-1}) were considered as explanatory variables, whereas for *B. microti* infections, which are chronic, a three-level covariate was used: uninfected, newly infected (infected at t_0 but not before), and chronically infected (first infected prior to t_0).

We found that this community of parasites represents not four independent infections but an interconnected web of interactions: Effects of other infections on infection risk were both strong and widespread, and connectance within the parasite community was exceptionally high, with evidence

detected for all possible pair-wise interactions (Fig. 1 and table S2). Both positive and negative associations were detected, and their magnitude was frequently considerable: increases up to a factor of 5.5 in risk and reductions in the odds of becoming infected on the order of 15% compared with uninfected individuals (Figs. 1 and 2). Indeed, perhaps most strikingly, in all cases, except for cowpox, infection with other parasite species explained more variation in infection risk than factors related to exposure risk and host condition, such as age and season (see relative changes in Akaike Criterion Information index (AIC) (13) in table S3). Moreover, the sizes of the effects of other parasites on infection risk were also similar to, and frequently greater than, other factors. For example, of all the noninfection variables, season generally had the largest effect on infection risk (table S3 and fig. S1), with seasonal increases in infection probability ranging from ~3 times as high (*A. phagocytophilum*) to 15 times as high (*B. microti*), but these were broadly matched by the magnitude of infection effects (Figs. 1 and 2). Effect sizes for noninfection variables are shown in fig. S1. These results are not explicable by simple co-occurrence of infections in hosts in poor condition, because for a subset of the data we explicitly account for variations in individual host condition indices at the time of infection (body condition and haematological condition (12), and there was no evidence of any reduction in the strength of between-parasite interactions (table S4). Thus, the most likely explanation for these effects is that interactions between these microparasites within individual hosts have a large impact on host susceptibility.

The effects of self-limiting infections were generally short-lived. Thus, although cowpox virus infection at t_0 consistently increased susceptibility to other parasites (by a factor of ~2) (Fig. 1), there was little evidence of effects of cowpox infection at t_{-1} , despite a correlation between infection status at t_{-1} and t_0 . Likewise, animals with ongoing *A. phagocytophilum* infections (infected at t_{-1} and t_0) were less likely to become infected with *B. microti*, but risk was not reduced in animals that had recently cleared an infection (infected at t_{-1} but not t_0) (Fig. 1). These observations highlight the dynamic nature of the immunological environment within hosts, and we speculate that susceptibility frequently returns to normal levels soon after clearance of the first infection.

Several infections increased susceptibility to other microparasite species. In such cases, release from effective control by the immune system is the most likely explanation, especially when supported by experimental studies. For example, laboratory studies have indicated the importance of immunomodulation for host exploitation by pox viruses (14), which may explain the positive effect of cowpox virus on susceptibility to other parasites. The same immune-mediated mechanisms might also account for our earlier demonstration that cowpox virus increases the length of *Bartonella taylorii* infections (10). Thus, mechanisms responsible for increasing susceptibility may also prolong infections in those that do succumb.

Strong decreases in susceptibility caused by other infections were also observed. The largest effect overall was reduced susceptibility to *Bartonella* spp. in individuals infected with *B. microti* and was especially apparent in chronically infected

Table 1. Summary information on the microparasite community, based on best available knowledge for infections in rodents.

Microparasite	Type	Mode of transmission	Infection length	Site of infection	Clinical signs/effects on fitness
Cowpox virus (CPXV) (19–21)	Virus	Direct	Self-limiting (4 weeks)	Respiratory tract and lymphoid tissues, mainly macrophages and monocytes	No obvious clinical signs but reductions in survival and fecundity
<i>Babesia microti</i> (22)	Protozoan	Ticks	Chronic (lifelong)	Erythrocytes, then sequestered in spleen and liver, periodic release into wider circulation	Hemolytic anemia but usually subclinical
<i>Anaplasma phagocytophilum</i> (23)	Bacterium	Ticks	Self-limiting (4 to 8 weeks)	Granulocytes, primarily neutrophils	Transient cytopenias
<i>Bartonella</i> spp. (24, 25)	Bacterium	Fleas	Self-limiting (4 to 8 weeks)	Vascular endothelium, followed by release and invasion of erythrocytes	None described

animals, where the odds of infection were 15% of those of uninfected animals (Figs. 1 and 2B). *B. microti* also decreases the length of *Bartonella taylorii* infections (10). Reciprocally, current *Bartonella* spp. infections were associated with reduced susceptibility to *B. microti* (Figs. 1 and

2C). Resource depletion may play a role here because both species target erythrocytes (Table 1). Reduced infection intensity of haemoparasites has been observed in mice coinfecting with anemia-causing helminths (3). Alternatively, negative effects may reflect up-regulation of mediators of a

cross-effective Th_1 response and therefore could represent an example of immunologically driven ecological interference (7, 15).

Our data also highlight the contrasting effects that one parasite may have on susceptibility to other parasites. *A. phagocytophilum* infection at t_0 increased susceptibility to cowpox by a factor of 5 (Figs. 1 and 2D) but substantially decreased susceptibility to *Bartonella* spp. (Figs. 1 and 2B). *A. phagocytophilum* infections are known to have immunomodulatory effects, provoking cytopenias and reducing numbers of both red and white blood cells (Table 1). Consequently, positive effects may be immune-mediated (down-regulation of immune response), whereas the reduced susceptibility to *Bartonella* spp. may also be immune-mediated (cross-effective response) and/or due to resource depletion.

We found, further, that whereas new and recently cleared *A. phagocytophilum* infections increased susceptibility to *B. microti*, ongoing *A. phagocytophilum* infections, as previously noted, decreased susceptibility to *B. microti* (Figs. 1 and 2C). There was also evidence in this case of reciprocal positive effects: Chronic and new *B. microti* infections increased the risk of *A. phagocytophilum* infection factors of 2 and 5, respectively (Figs. 1 and 2A). Both of these microparasite species are tick transmitted, and the co-occurrence of tick-borne pathogens is common (16). Despite the inclusion of tick abundance in the models, the reciprocal positive interactions could be a case of unaccounted-for correlations in exposure risk such as coinfecting ticks (17), whereas the negative effect of ongoing *A. phagocytophilum* infection could reflect an antagonistic interaction within hosts. For example, the suppression of *Babesia divergens* by simultaneous *A. phagocytophilum* infections has been observed in cattle (18).

Finally, our results emphasize that the standard practice of classifying individuals in natural

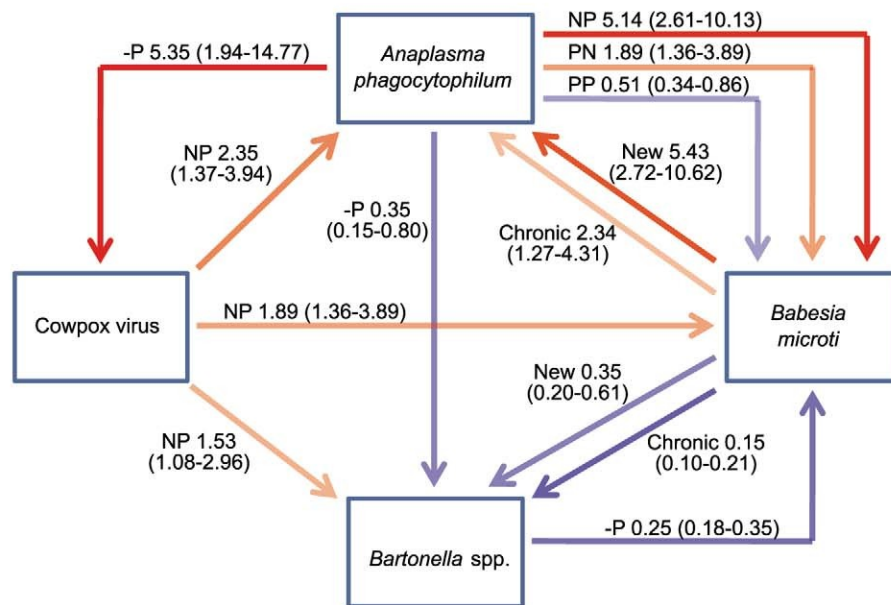


Fig. 1. The web of interactions between microparasite species within this community, showing the magnitude of effects. All associations shown obtained overwhelming support (accumulative weight in models >0.9) (see table S3). Positive associations are shown in red [odds ratio (OR) >1] and negative associations (OR <1) in blue, with the strength of the line color reflecting the magnitude of the effect. ORs (exp β) (see table S3) relative to uninfected individuals, with 95% confidence intervals in brackets. Thus an individual with a new *B. microti* infection is ~5 times as likely to become infected with *A. phagocytophilum* than an uninfected individual (OR = 5.43). Infection history associated with each effect is also indicated: N, negative, P, positive. Thus, NP indicates no infection at t_{-1} , infection at t_0 . -P is used to signify that NP and PP show similar effects. Because *B. microti* induces a chronic infection, there are three infection status histories (uninfected, chronic, and new infections). For cowpox virus, a probability of infection was used in analyses, and results are for individuals that seroconverted at t_0 (i.e., NP infection history, probability of infection at $t_0 = 0.5$) (12).

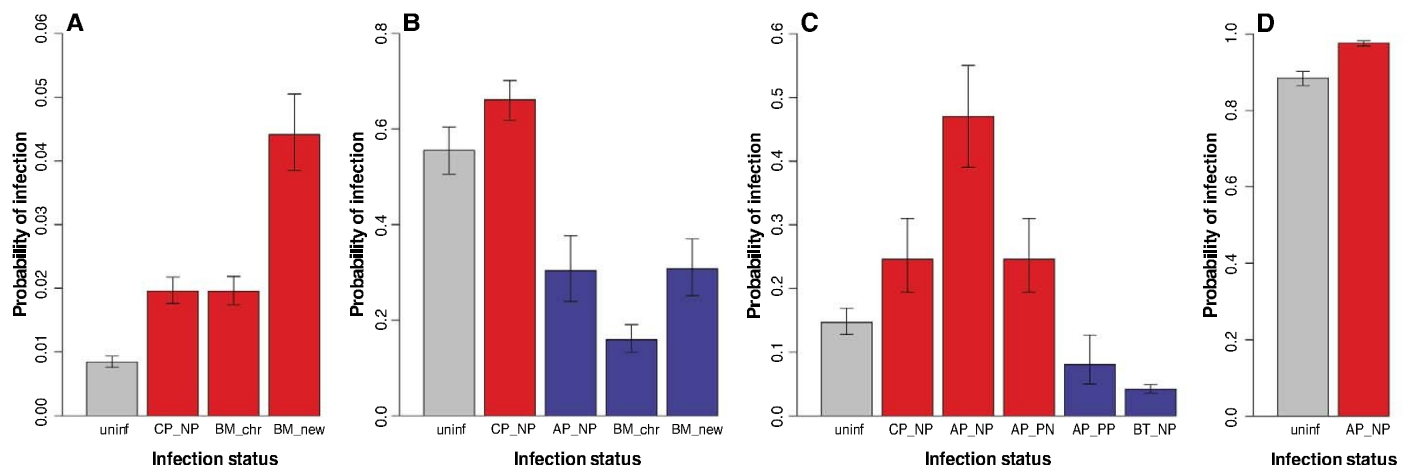


Fig. 2. Predicted probabilities of acquiring an infection depending on infection status by other parasites for (A) *A. phagocytophilum* (AP), (B) *Bartonella* spp. (BT), (C) *B. microti* (BM), and (D) cowpox virus (CP). Predictions are based on the models in table S3. Gray bars represent individuals without other infections (uninf); red and blue bars highlight positive and negative effects on susceptibility, respectively. Each bar is labeled with

the infection (e.g., CP) and the infection history (see Fig. 1 for explanations) associated with each effect. Error bars represent 95% confidence intervals, averaged over random effects. Predicted probabilities are defined with reference to an 18-g male in July at one specific site. In cases where NP and PP show similar effects, predicted probabilities are based on an NP infection history.

populations as infected or uninfected by one parasite alone fails to recognize that much more may be implied by the categorization “infected.” For example, cowpox virus infection has been associated with major reductions in survival and fecundity (19, 20). However, in our results, 39% of those infected with cowpox virus were also infected with *B. microti* ($n = 651$), 65% of the remainder had *Bartonella* spp. infections ($n = 396$), and overall 79% were coinfecting with at least one of the three microparasites considered. Clearly, even when substantial associations between a given infection and host fitness are detected in a wildlife host, attributing the effect to that parasite alone may be unjustified.

This study demonstrates that communities of microparasites may be structured by strong interactions between species, consistent with past cross-sectional studies that have highlighted associations between parasite species (7, 8). In particular, by using time-series data, we provide the first evidence for microparasites in natural populations that such interactions can be driven by effects on susceptibility and can have as much impact on infection risk as more commonly considered factors such as host age and season. Because field voles are also infected by macroparasites, as well as other microparasites, our study only considers a portion of possible interactions in the parasite community. For instance, macroparasites and microparasites apparently interact negatively in natural populations of African buffalo through accelerated mortality and immune-driven effects on susceptibility (15). Thus, parasite community interactions in voles may well be even richer than

those we have measured. Such strong effects on individual host susceptibility are likely to directly influence population-level parasite dynamics and may have profound consequences for disease management programs. Specifically, where interactions are antagonistic, control that targets one parasite species may result in unexpected increases in a second parasite species. To predict and control parasites and disease in natural populations, we need to understand community interactions among parasites and not just single host-parasite interactions.

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Supporting Online Material

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Materials and Methods

Fig. S1

Tables S1 to S5

References

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Great science forms the building blocks of any quality R&D-based company. But superb internal communication serves as the glue that binds those building blocks together and makes for a top employer, say managers with the top companies recognized by *Science* this year. The top employers in this year's survey all take pains to hear employees' concerns, recognize everyone's contribution toward overall goals, and keep staff informed of how external events—like last year's global financial crisis and climate of mergers and acquisitions—will or won't affect them.

Only two of the 2010 top 10 didn't make the list last year—Millennium: The Takeda Oncology Company and Vertex Pharmaceuticals Incorporated (see list on page 250). However, Millennium made the list in 2007 and 2008. Over the last five years the composition has changed from less Big Pharma to include more Big Biotech—including perennial leader Genentech, a member of the Roche group, which has held the number one spot eight out of nine years (the exception was 2007, when Boehringer-Ingelheim claimed the lead).

In first place again this year, Genentech has consistently ranked high, as well as being recognized as a great employer by *Fortune*, *Forbes*, and other publications because it has a research focus emphasizing patients' needs combined with treating employees with respect, says **Marc Tessier-Lavigne**, executive vice president for research and chief scientific officer of Genentech. "Robert Swanson, one of the company's founders, always said, 'Our most important asset goes home every night in sneakers,'" says Tessier-Lavigne.

Research innovation and respect for employees remain the most important criteria for being a top employer, according to survey respondents. And, the top employers report that open communication is the best strategy for gaining employees' respect, fostering creativity, and navigating tough times.

RESPECT AND LISTENING

Creating a culture of respect requires true two-way communication between employees and managers. This year's top employers use a variety of communication strategies—from "town halls" to employee feedback about managers—to ensure that workers' concerns are heard. To be truly effective, the style of communication must reflect the culture of the company.

As Vertex approaches the commercialization of its first drug, the company faces many new communication challenges, including the tremendous growth across the organization. "We're establishing

Creating a culture of respect...

...requires true two-way communication between employees and managers.

the company's first commercial function, which includes the hiring of our first sales force," says **Lisa Kelly-Croswell**, senior vice president, human resources. With currently more than 150 openings globally and having hired more than 200 employees in 2009, Vertex works on providing transparent communication during this rapid growth period by holding monthly town hall meetings and offering a two-day course on "The Business of Vertex," which helps employees understand business principles and addresses the many internal and external issues of being a public company, says Kelly-Croswell.

No question is "out of bounds" at town hall meetings, says **Steve Gansler**, senior vice president, human resources at Millennium: The Takeda Oncology Company, which holds the number four spot this year. They, too, encourage employees to share their opinions openly during monthly meetings, and afterward attendees receive surveys that ask whether the presentations met their needs, says Gansler. People keep returning to the non-mandatory meetings; Gansler suspects that their efforts for real two-way communication explain the high attendance. **Cont. on p. 250 »**

UPCOMING FEATURES

Developing a Career Plan (online only)—December 3

Diversity: Barriers for Women Scientists—January 21

Faculty: Moving Up The Academic Ladder—February 11

TOP EMPLOYERS SURVEY



TOP TWENTY EMPLOYERS

2010 Rank	2009 Rank	Employer (Global Headquarters)	Innovative leader in the industry	Treats employees with respect	Has loyal employees	Is socially responsible	Clear vision	Does important, quality research	Work & personal values are aligned
1	1	Genentech (South San Francisco, CA)	•		•		•		
2	4	Monsanto Company (Creve Coeur, MO)	•			•	•		
3	–	Vertex Pharmaceuticals Incorporated (Cambridge, MA)		•		•			•
4	5	Millennium: The Takeda Oncology Company (Cambridge, MA)		•			•	•	
5	17	Roche (Basel, Switzerland)	•		•		•		
6	2	Boehringer Ingelheim (Ingelheim, Germany)	•		•		•		
7	20	Syngenta (Basel, Switzerland)		•			•		•
8	3	Genzyme Corp. (Cambridge, MA)			•	•		•	
9	6	Merck KGaA/Merck Serono/EMD Serono (Darmstadt, Germany)			•	•		•	
10	8	Amgen (Thousand Oaks, CA)	•			•		•	
11	12	Novartis (Basel, Switzerland)	•				•	•	
12	10	Johnson & Johnson (New Brunswick, NJ)				•	•	•	
13	–	Abbott (Abbot Park, IL)			•		•	•	
14	11	Eli Lilly and Company (Indianapolis, IN)			•			•	•
15	15	Biogen Idec (Weston, MA)	•	•				•	
16	16	Merck (Whitehouse Station, NJ)	•			•		•	
17	9	Gilead Sciences (Foster City, CA)	•					•	•
18	19	sanofi-aventis (Paris, France)	•				•	•	
19	–	DuPont (Wilmington, DE)		•	•			•	
20	14	AstraZeneca PLC (London, UK)			•		•	•	

SURVEY METHODOLOGY

The top companies were identified through a web-based survey, conducted from April 13–30 by Brighton Consulting Group and Cell Associates. Fifty-seven percent of the total qualified survey respondents came from the initial e-mail invitation to 80,000 individuals worldwide. An additional 1,000 e-mails sent specifically to human resource contacts from the *Science* Careers sales database, comprised the remaining 43 percent of the qualified respondents.

Survey takers then rated the companies they had chosen on 23 driving characteristics. The top 20 companies were selected using a statistical process that calculates a unique ranking score for each company. Only companies that were rated by 30 or more respondents were eligible to become part of the top 20 employers.

The 20 companies with the best reputations as employers and the top three driving characteristics for each company, according to respondents in the 2010 survey undertaken for the AAAS/Science Business Office. The companies without a 2009 rank did not rank among the top 20 in the 2009 survey.

"We try to keep hierarchy and bureaucracy to a minimum," Genentech's Tessier-Lavigne says. That means maintaining a true "open-door" policy to both hear employee opinions and explain the company's direction. "Not everyone's first choice will be the choice we go with," explains Tessier-Lavigne. "But we try to explain the rationale when we go in a particular direction, and how and why the decision was made."

The level of candor tends to be higher in smaller group settings, posits **Alan Smith**, senior vice president of Genzyme, which holds the eighth position. He "gauges the temperature" of employees through weekly lunches with six people from all around the company. Smith says Genzyme also holds town hall meetings, but "it's different from when it's six people at lunch." He and his lunch mates chat for about 90 minutes about company issues, and he goes through his decision-making process. "At lunch they feel freer to say what they like," explains Smith. This type of honesty fosters respect in an organization, he says. Constructive criticism of science is also a part of that honesty and openness. The company holds weekly "Science Peer Review Group" meetings, with that aim. "We remind the scientists that we are critiquing the data, not criticizing them," Smith says.

Syngenta, which moved up 13 places since last year's survey to seventh, recruits employee input for more than science and business. They aim to make two-way communication concrete—literally, says **Michiel van Lookeren Campagne**, Syngenta's head of global biotechnology. "When we moved into a new building in April this year, our employees helped with the design. Employees also help design our processes," he explains.

ACCOUNTABILITY

To engender respect, there needs to be accountability, but respect is an abstract quality. However, Vertex—which makes its first appearance on the top employer list this year at number three—turns it into a reality by evaluating employees not only on their performance, but also on how they measure up to the company's values. Those values include treating employees with respect—of which a large component is how to communicate with different kinds of personalities throughout the company, says Kelly-Croswell. To help employees meet this goal, Vertex requires all employees to complete a course called "The Art of Speed-Reading People," based on the Myers-Briggs Type Indicator test. The course teaches people to **cont. on p. 252**

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Lynn, Patient

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In October 2010, Genentech was named "top employer in the biopharmaceutical industry" by Science magazine.



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Sara Kenkare-Mitra, Ph.D.

Vice President, Development Sciences
12 years with Genentech

"Diseases of the brain such as Alzheimer's disease and schizophrenia cause immeasurable suffering to countless people throughout the world. Effective treatments can only be discovered by excellent innovative science. For me, "Life Inspired" represents the dedicated scientists at Genentech who come to work every day eager to apply their expertise, creativity and team spirit to making a difference for patients."

Morgan Sheng, Ph.D.

Vice President, Neuroscience
Two years with Genentech



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TOP EMPLOYERS SURVEY



SURVEY DEMOGRAPHICS

GENDER

58% male, 42% female

EXPERIENCE

65% have 10 years or more work experience

CAREER STATUS

74% report that they have not yet reached the peak of their careers

COMPANY TYPE

48% biotech, 37% pharma, 7% university, 8% other: more than 4 out of 5 work in private industry

GEOGRAPHY

72% from North America; 22% from Europe; 5% from Asia/Pacific Rim; 1% from rest of world

understand what their personality type is and how they communicate and interact. It also helps employees recognize and interact with communication styles that are different from their own.

Appraisals can go both ways. Monsanto Company, which rose to number two this year after appearing fourth last year, has employees rate their managers in an annual survey called “leadership 180.” This helps managers gauge their own performance through the eyes of their team, says **Robb Fraley**, Monsanto’s executive vice president and chief technology officer.

Creating this culture of respectful two-way communication is taken seriously, says **Bernd Kirschbaum**, head of Global R&D, Merck Serono (Merck Serono and EMD Serono are part of Merck KGaA, which is ranked ninth). The company emphasizes the team over the individual, which means taking action when respect for either individuals or the team are lacking, he says. “We have discontinued contracts of people who have not embraced our values—for instance, managers who were rude or abusive to the employees they supervised,” explains Kirschbaum. “If you ignore this as an organization and as a leader, you completely lose credibility. You need to say, ‘This is unacceptable.’ You need to follow up.”

ENCOURAGING INNOVATION

Open communication builds respect and a harmonious work place, but letting employees know that their role in the company is meaningful, their research is cutting-edge, and that creativity is valued is how many of these top companies stay positioned at the forefront of scientific discovery and at the top of *Science’s* survey.

With the look and feel of a university campus, Genentech has consistently created such a culture. But that culture is more than superficial. For instance, the popular postdoc program at Genentech fuels the innovative environment, both directly and indirectly. Postdocs make up about 10 percent of the company’s research community.

They are not attached to “pipeline” projects; rather, they are free to do basic research. Their goal is to do cutting-edge work that will merit publication in a top-tier journal. “It brings creative and energetic individuals into the organization,” says Tessier-Lavigne. “They challenge the status quo.” These types of positions allow scientists to follow their noses in more basic research areas, which might yield findings that the company can later capitalize on.

Challenging the status quo, for Genzyme, means seeking treatments for unmet medical needs. “We don’t do ‘me too [drugs],” says Smith. The company recognizes that ideas will fail more often than succeed—what looks like a good idea on paper is not always a good idea in patients. “We’re forgiving as an organization,” Smith says. “We don’t kill ourselves with angst over things that don’t work.” However, having high goals is a key component of being an innovation leader, says **Roger Perlmutter**, executive vice president of research and development at Amgen, which placed tenth this year. “We focus on grievous illness,” Perlmutter says. “You could say ‘of course you do.’ But many companies seek modest improvements in existing therapies. We focus almost exclusively on programs where there have been few or no drugs developed.”

Another way companies encourage innovation is by offering incentives. Several of the top employers use contests to up the ante. Merck KGaA actively solicits new ideas internally, says Kirschbaum. “The whole company is asked to provide ideas that they normally wouldn’t get support for,” Kirschbaum says. A committee picks the winners and the awardees get to run the project as if they were entrepreneurs. Vertex holds a company-wide contest for an “outstanding research award” of \$10,000 to a person who is recognized by senior leaders and peers. And to encourage new thinking, Vertex started an internal exchange program last year that they call “step out.” The winning applicant is able to explore a research area outside of their own expertise for three months. Millennium’s “success share” program encourages innovation by linking a portion of employee bonuses to the achievement of annual corporate and individual goals, including product revenues and advancement of the pipeline.

Finding fresh ideas can go beyond internal contests to friendly competition. Roche, which rose to fifth place this year from seventeenth in 2009, has three independent pharmaceutical research and early development units in-house to lay the foundation for innovation through diverse approaches. “The way each of these units tackle challenges and the resulting outcomes can be very different,” says **Sylvia Ayyoubi**, global head of human resources. However, Roche also searches outside the organization for ideas, with around 150 actively managed research partnership globally. Monsanto reaches into an even larger reserve of research collaborations—with over 1,500 partnerships between academic institutions and public and private organizations—for advances that always start with the concept that any new idea must help farmers better raise a crop, says Fraley.

One such agreement is a “yield and stress” collaboration with BASF, announced in 2007, where both companies jointly agreed to put \$1.5 billion into developing crops that withstand various biotic and abiotic stresses and have higher yields. In July, both companies also committed to contribute up to another one billion dollars. Monsanto also uses grants and scholarships to fuel their innovation, including the \$10 million Beachell-Borlaug International Scholars Program for research in rice and wheat that was announced last year.

Seeing the results of an innovative approach serves as the ultimate motivation for scientists, says Syngenta’s Campagne. This year the company launched a new model for insect control developed from start to finish at its Research Triangle Park, **cont. on p. 254 »**

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TOP EMPLOYERS SURVEY

DRIVING CHARACTERISTICS

2010

1. Innovative leader in the industry
2. Treats employees with respect
3. Loyal employees
4. Socially responsible
5. Clear Vision
6. Does important quality research
7. Work and personal values are aligned

2009

1. Innovative leader in the industry
2. Treats employees with respect
3. Loyal employees
4. Socially responsible
5. Does important quality research
6. Leadership moves company in right direction
7. Work and personal values are aligned

"People feel that we are contributing to making the world a better place."

Black type indicates the characteristics in common for the two years.

North Carolina site and was recently approved by the FDA for use in the United States in 2011. Next year, Syngenta will launch a project to find ways to grow crops with less water. The knowledge that they are making a difference serves as excellent motivation. "People feel that we are contributing to making the world a better place," says Campagne.

TRANSPARENCY

It's easy to keep employees happy in periods of prosperity and stability—when products cruise through the pipeline, the stock market soars, and the company finds itself flush with capital. 2009 was not that time. But this year's top employers excel at keeping attitudes and innovation high in the face of adversity. The global financial crisis, mergers and acquisitions, uncertainties about the U.S. health care policy, and ongoing controversies about genetically modified organisms (GMOs) were all factors that impacted which employers were rated highly in *Science's* survey. As with fostering an environment of innovation and creating an atmosphere of respect, these top companies also find communication key for heading off these challenges.

• THE FINANCIAL CRISIS

The global blows that the stock market took in 2009 meant that some companies had less to spend on R&D and salaries. But communicating the problem and asking colleagues to help in the solution bolstered spirits at both Merck KGaA and Genzyme last year.

Merck KGaA's Kirschbaum says the company prides itself in putting 20 percent or more of its total revenue back into R&D. But last year, with revenues down, that goal was threatened. The company had to either to stop some development projects or come up with \$80 million from somewhere else. "There was great solidarity from our commercial colleagues in the sales, marketing, and PR side of the company, who helped us find and invest money from their budget," Kirschbaum says. They decided that R&D was important for the long term health of the company and in the short term it should trump marketing, business development, and other commercial activities. "The support from the commercial side was very rewarding for the self-confidence of the R&D people."

Last year, Genzyme had to slow down its rate of recruitment, says Smith. "That is not something that anyone wants to hear; for junior faculty and junior staff, it's not welcome news," says Smith. Working to keep the lines of communication open, Smith had to let em-

ployees know that, "we simply can't afford to hire new people right now." However, he also reminds employees that there are ways to get the required staff if they are at a key point in a project. "If we decide that position is necessary, we try to transfer people to that project, or if someone has recently left the company then we may be able to hire a new staff member."

• MERGERS AND ACQUISITIONS

There are multiple ways of dealing with a merger, whether you are the acquirer or the acquiree. Companies can become part of a new seamless entity, retain their independence, or just be mined for their scientific pipeline or technological tools. There are no right ways, but establishing a new culture or reinforcing an existing one is key, as is making the process as transparent as possible, so employees know how any realignment will affect them.

Roche's Ayyoubi says transparency, as well as fast and reliable information, help build trust and keep employees committed—especially in times of change such as during the merger with Genentech. "We tried to be very clear about the terms and conditions, and which sites would be affected." The company used an extranet, multiple town halls, and lunches with senior management to spread information.

When Roche made its bid in July 2008 to fully acquire Genentech, facilitated by a financial stake in the company since the early '90s, it announced that priority number one was to preserve the Genentech culture, says Tessier-Lavigne. As a result, both Genentech and Roche have their own autonomous research and early clinical discovery tracks. The head of Genentech Research and Early Development, executive vice president Richard Scheller, reports directly to Roche CEO Severin Schwan, not through Roche Research. Genentech's academic atmosphere, work hard/play hard culture, and strong postdoc program have all been preserved following the March 2009 acquisition. For these reasons, *Science* chose to list Genentech and Roche separately in this year's survey. "As a result of that pledge to allow Genentech to remain independent, not a single scientist has left the organization because of the merger," says Tessier-Lavigne. "And we have been able to recruit additional senior leadership through the merger." One exception, however, is Tessier-Lavigne himself, who will be moving to Rockefeller University in March 2011.

In Millennium's case, being acquired has meant more resources. Takeda Pharmaceuticals bought the company in **cont. on p. 256** »

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TOP EMPLOYERS SURVEY

2008, but like Roche with Genentech, kept the company's culture intact. Since being acquired, Millennium has added more than 300 positions because the parent company recognized the Millennium's strength in cancer treatment R&D. "Takeda transferred its entire oncology program to us," Gansler says.

Unlike Genentech, Millennium has been more fully integrated, making researchers more likely to work with their counterparts at the parent company. "By being part of a larger organization, we have been the beneficiary of significant investment that perhaps wouldn't have happened had the acquisition not occurred," explains Gansler.

Other companies that have acquired smaller ones must work hard to ensure a seamless transition, while also reassuring their employees that they won't, in turn, be acquired. Merck KGaA, which recently completed its acquisition of Millipore, reminds its own employees that they will never be a part of an acquisition since the Merck family, which founded the company over 300 years ago, still owns 70 percent of it. "This gives employees a sense of stability," says Merck KGaA's Kirschbaum. Instead, Merck KGaA looks for complements to its existing R&D strategy. "The Millipore acquisition helps both the chemistry and pharmaceutical divisions," Kirschbaum says. He feels that acquisitions made when a company's pipeline dries up or blockbuster products go off patent are bad for morale. This appears to reflect a failure on the parent company's long-term R&D strategy. "We don't want to be put in that position where we have to buy another company because we have nothing in our own pipeline; it makes employees nervous."

Genzyme, too, has a history of making acquisitions to augment its own research efforts. "We have a way of embracing new ideas, new technology, and new staff," says Smith. Since his own position at Genzyme came about due to an acquisition, Smith can empathize with employees brought in when a company goes shopping for new technology. "I, myself, was at another company for four years (Integrated Genetics) when Genzyme acquired them," says Smith. "So I can say 'I know how you feel.'"

• GENETICALLY MODIFIED ORGANISMS

AgBio companies, three of which made the 2010 top 20 employer list including DuPont, Monsanto, and Syngenta, use genetically modified organism (GMO) technology to improve crop yield and decrease dependence on irrigation and pesticides. However, because some public skepticism remains about the use of GMOs, the company puts a lot of effort into communicating, to both the public and their employees, why such techniques are both safe and necessary, says Fraley at Monsanto. Acceptance of GMO technology begins with

the scientists in-house, says Campagne, at Syngenta, "Everyone who works in science knows that we have created safe and very good products that help sustainable agriculture," Campagne says. "The scientists working for us are 100-percent convinced that this is the right thing to do," he says.

The company also works to share this technology with parts of the world that are unable to afford it through partnerships with non-profits. In 2008, Monsanto partnered with the African Agricultural Technology Foundation, CIMMYT (the international maize and wheat development center),

and the Bill & Melinda Gates and Howard G. Buffett foundations for the Water Efficient Maize for Africa (WEMA) project. The goal is to help make drought-tolerant corn available to farmers in sub-Saharan Africa at approximately the same time as its commercial launch in the United States.

• HEALTHCARE REFORM

The exact cost of health care reform in the United States remains unclear—for both companies and consumers. Rather than worrying about it, some companies have taken an active role in advocating for it—letting both employees and customers know that the company cares about access to health care. "We took a leadership position on health care reform," says Amgen's Perlmutter. "It really is important that Americans have access to health care. We say this not because it will be a big profit center for us, but because it's the right thing to do."

Merck KGaA, too, became involved with health care reform. The company wanted to help shape the legislation, rather than just have to react to it, and contributed its global perspective to the dialogue, says Kirschbaum. "Different countries have different dynamics. We try to engage in a dialogue despite the challenges," explains Kirschbaum. "We have an ethical obligation to bring compounds to the market to help people with very serious diseases. But we need to make sure that this endeavor remains sustainable."

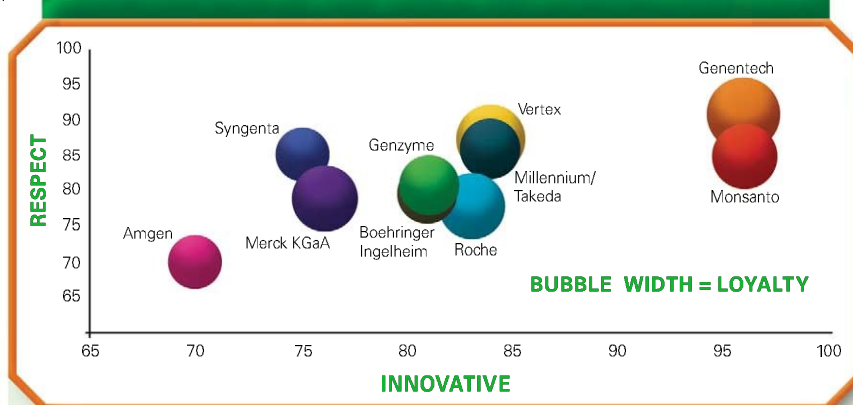
Effective communication, both in times of success and adversity, generates another kind of sustainability—quality employees who stick around and produce the innovative scientific discoveries that define top employers. Treating employees with respect and keeping two-way communication open during triumphs and challenges is perhaps the best way for employers to be respected—both by their employees and by their peers.

Paul Smaglik is a freelance writer living in Milwaukee, Wisconsin.

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COMPARISON OF TOP TEN'S TOP CHARACTERISTICS



Comparison of the top 10 companies on the basis of the top 3 drivers (scored out of 100): Loyalty (bubble width), Innovative leader (x-axis), and Treats employees with respect (y-axis).



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Requirements:

- Ph.D. in Molecular Biology, Biochemistry or relevant biological science, 2-5 years experience in protein expression and engineering
- Solid theoretical basis and hands-on experience within molecular biology, special focus on expression of proteins in *E. coli*.
- Experience from pharmaceutical industry with *E. coli* process development, fermentation and handing over to CMC setting is an advantage

- Senior/Principle Scientist, Protein chemistry and process development [Job Code: DR_201009_PPPD]:

Responsibility:

You will contribute to several early stage projects with expertises within protein purification, handling and characterization. Furthermore, you will be responsible for developing protocols for protein refolding, initial formulation, process development and transfer of methods into development settings. You will also take part in developing new approaches for analysis of chemical structure and stability applying state-of-the-art liquid chromatography and mass spectrometry. Insight within protein design is an advantage.

Requirements:

- Ph.D. in protein chemistry, proteomics, biochemistry or related discipline, at least 2-5 years experience in protein purification and characterization
- Solid theoretical basis and hands-on experience as well as an excellent track record of original research.
- Experience from pharmaceutical industry with protein purification and process development and handing over to CMC setting is an advantage

Furthermore, we also have 3 positions open within Biopharmaceutical Research Unit:

- Scientist/Senior Scientist, Antibody Phage Display [Job Code:MB_201009_Phage]
- Scientist/Senior Scientist, Fermentation technology [Job Code:MB_201009_Fermentation]
- Scientist/Senior Scientist, Cell Biology [Job Code:CB_201009_Sci]

For full description of requirements, please visit: <http://www.ebiotrade.com/jobf/readall1.asp?cmpidn=y2003527103359>.



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Right from the beginning, I've had
the freedom to come up with new ideas,
have them listened to, build something from
scratch and run with it."*

Tim J.
Roche, Switzerland



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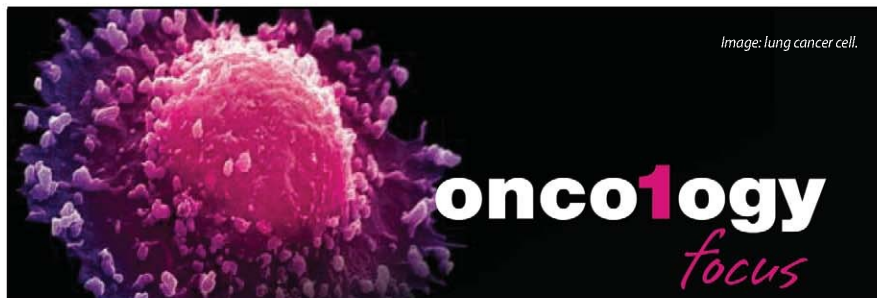


Image: lung cancer cell.

oncology focus

One focus: a shared commitment to improve the lives of cancer patients worldwide.

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CHIEF EXECUTIVE OFFICER

World leading tropical marine research



Australian Government



AUSTRALIAN INSTITUTE
OF MARINE SCIENCE

The Australian Institute of Marine Science (AIMS), the nation's tropical marine research agency, seeks a new leader at a time of unprecedented opportunity for the Institute.

Established to provide marine research in support of Australia's extensive marine estate, AIMS is a world-class, high-profile organisation at the forefront of tropical marine science. It is an exciting and innovative research hub operating on the international stage, with research programs addressing areas as diverse as climate change, sustainable development, discovery science and marine observing systems. Assets include two large research vessels, state of the art laboratory facilities, a headquarters near Townsville, Queensland, and major laboratories in Darwin and Perth. The Institute is in the midst of a once in a generation \$55 million re-development, including the construction of a groundbreaking \$32 million seawater experimental facility in Townsville and partnering in the new \$63 million Indian Ocean Marine Research Institute in Perth. AIMS is a Commonwealth Statutory Authority, with a staff of 220 and an annual budget of \$50 million.

The Position:

- Leadership at both national and international levels
- Provide strategic direction and vision for the large, highly skilled multi-disciplinary research and support teams
- Strengthen relationships with industry, Government, academia and other stakeholders to build support for marine research
- Maximise the impact, uptake and growth of marine science in Australia through collaborations and partnerships

The Person:

- Ability to build and maintain effective consultative links with local, regional and national representatives of Government, Industry, academia and the wider community
- A successful track record in attracting financial support
- Leadership, management and communication skills to engage with AIMS' vibrant community of research and support professionals.
- PhD qualified (or equivalent) with at least 10 years experience in marine science and a track record of achievement

Please visit www.first-place.com.au 'Opportunities' for a position brief. Select 'How to Apply' to submit your application. Confidential enquiries to Peter Gibson or Louise Baker on +61 7 3368 5300. Closing Date: Monday October 18, 2010

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University of
Central
Florida

Two Assistant Professorships in Biological Sciences

The Department of Biology at the University of Central Florida (UCF) invites applications for two tenure-track positions at the Assistant Professor rank. For Position Number 38667, we seek applicants who use innovative approaches to address **evolutionary or environmental** questions in **genetics, cell biology, or development**. For Position Number 38942, applicants must have a research focus in a sub-discipline of **conservation biology**. Preference will be given to candidates who add to and complement current faculty research and teaching strengths: see biology.cos.ucf.edu. Successful candidates for both positions must have a Ph.D. in a relevant field, appropriate post-doctoral training and a demonstrated ability or strong potential to establish and maintain a vigorous, extramurally funded research program. S/he will contribute to our Ph.D. programs in Conservation Biology and/or Biomedical Sciences and M.S. program in Biology, and teach graduate and undergraduate courses. UCF has a strong research emphasis and provides competitive startup funds and teaching loads.

Applicants must complete an online job application at: www.jobswithucf.com and separately e-mail a **single** PDF document that includes a letter of intent, curriculum vitae, a list of three references with their contact information, and statements of research plans and teaching philosophy to: bio-search@mail.ucf.edu. Please include the relevant position number in the subject line. The letter of intent should explain how your research will complement current faculty research areas. Review of applications will begin **November 15, 2010**, with an anticipated start date of August 2011.

The University of Central Florida is an Affirmative Action/Equal Opportunity Employer. Minorities and women are encouraged to apply. As an agency of the State of Florida, all application materials and selection procedures are available for public review.



Postdoctoral and Predoctoral research positions are open in the cryo-electron tomography group at the Goethe University Frankfurt. The research is funded by the European Research Council (ERC) and the European Commission seventh framework programme (EU FP7).

The group of **Dr. Frangakis** (www.biophysik.org/frangakis/) at the Frankfurt Institute for Molecular Life Science is an interdisciplinary team of biologists, physicists, and computational scientists. We use cryo-electron tomography to visualize biological systems, and provide new insights into the three-dimensional ultrastructure of eukaryotic cells. Our images can achieve molecular resolution *in vivo*, which allows us to generate a quasi-atomic atlas of supramolecular structures.

The lab is equipped with latest technology electron (FEI Titan Krios) and light microscopes as well as state-of-the-art accompanying equipment.

Investigation of the chromatin structure

Aim of the project is to study the structure of the chromatin fiber and elucidate its role in fundamental biological processes such as transcription or silencing. Despite its importance and the effort over three decades, the structure of the 30nm chromatin fiber remains controversial. Towards this goal a combination of *in vitro* and *in vivo* samples will be used.

Investigation of adhesion junctions in mouse models

Aim of the project is to structurally characterize adhesion junctions *in toto* by cryo-electron tomography of vitreous sections. Adhesion junctions are clusters of membrane-associated proteins that are involved in intercellular contacts. We aim to reveal the molecular organization of several adhesion junctions, and their implications in cellular proliferation and disease.

The successful candidates should be highly motivated and have a profound knowledge in Cell Biology, Biochemistry, Biophysics or related fields. First experience in electron microscopy would be an advantage. The projects are funded by the Marie Curie ITN program Nucleosome4D and the ERC. Applicants must be nationals of an EU Member or Associated State, or have resided in the EU for at least five years immediately prior to their selection.

Interested candidates should send an application including a cover letter stating areas of expertise and interests, a CV, publication list and names and contact information for 3 references to **Dr. Achilleas Frangakis** (e-mail: cryo@biophysik.org).



Faculty Position Department of Molecular Biology Princeton University

The Department of Molecular Biology at Princeton University invites applications for a tenure-track faculty position at the assistant professor level. We are seeking an outstanding investigator in the area of biochemistry and structural biology. We are particularly interested in candidates whose plans to address fundamental biological questions include the use of X-ray crystallography. Applicants must have an excellent record of research productivity and demonstrate the ability to develop a rigorous research program. All applicants must have a Ph.D. or M.D. with postdoctoral research experience and a commitment to teaching at the undergraduate and graduate levels.

Applications must be submitted online at <http://jobs.princeton.edu>, requisition #1000770, and should include a cover letter, curriculum vitae and a short summary of research interests. We also require three letters of recommendation. All materials must be submitted as PDF files. For full consideration, applications should be received by **December 1, 2010**.

Princeton University is an Equal Opportunity Employer and complies with applicable EEO and affirmative action regulations.



The Department of Pharmacology & Experimental Therapeutics at

Boston University School of Medicine (<http://www.bumc.bu.edu/busm-pm>) has an open faculty position in neuroscience for a scientist or physician scientist at the Assistant, Associate or Full Professor levels. Individuals with an interest in basic or translational sciences (including clinical trials), bridging clinical and basic disciplines such as psychiatry, neurology, biomedical engineering, systems biology, bioinformatics, cancer or cardiovascular pharmacology, are encouraged to apply. The Department has strengths in a broad range of research areas including substance abuse, gene therapy, learning and memory, neuropeptides, neurodegenerative diseases, neuroinflammation, anxiety, and epilepsy. The Department administers an active university-wide pharmacological sciences training program in Biomolecular Pharmacology that is supported by an NIGMS T32, awarding a PhD in Pharmacology and Experimental Therapeutics or combined degrees in Pharmacology-Neuroscience or Pharmacology-Cell & Molecular Biology. Joint appointments with clinical departments such as Neurology, Psychiatry, or Medicine are encouraged for physician-scientists seeking some clinical service opportunities. Please send a CV, description of future research, and up to three peer-reviewed publications electronically to: **David H. Farb, Ph.D., Professor and Chair, Department of Pharmacology & Experimental Therapeutics, Boston University School of Medicine, 72 East Concord Street, L-603, Boston, MA 02118 at Pharm3@bu.edu.**

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University of
Massachusetts
UMASS Medical School

Diabetes

CENTER OF EXCELLENCE

FACULTY POSITION

The Diabetes Center of Excellence at the University of Massachusetts Medical School invites applications for a **SENIOR TENURED** or **JUNIOR TENURE-TRACK** basic science or clinical investigator faculty position. The Diabetes Center of Excellence currently consists of basic and physician scientists representing a broad range of disciplines in the biomedical and clinical sciences, with members from several Medical School Departments and Programs working together as central New England's leading center for diabetes clinical care, innovation, and discovery. The Center occupies state-of-the-art research space on an expanding Medical School campus, and benefits from being a continuously funded Diabetes-Endocrinology Research Center (DERC) for over 28 years. Basic investigators benefit from core facilities for deep sequencing, proteomics, genotyping, fluorescence-activated cell sorting, digital imaging/confocal microscopy, genomics/bioinformatics, transgenic/knockout mice, and mouse metabolic phenotyping. Clinical and community investigators benefit from infrastructure for recruitment and retention of research participants, measurement, behavioral and nutritional intervention resources and facilities, and academic-community research partnerships. Adult and pediatric clinical efforts of the Diabetes Center are housed in a new Ambulatory Clinical Care building designed so that patients can receive appropriate retinal photos, foot care, diabetes education, and other routine medical care at one location. The position will be highly competitive with regard to start-up funds, laboratory space and salary. The Center seeks an individual of outstanding research potential relating to diabetes pathophysiology or treatment.

Applicants should send curriculum vitae, statement of research interests, and names and addresses of three references to:

Dr. David M. Harlan
Co-Director, Diabetes
Center of Excellence
Search Committee Chair

% Patricia Cannon
55 Lake Avenue North
Ambulatory Care Building,
Room AC4.127
Worcester, MA 01655

Patricia.cannon@umassmed.edu
508.856.3800

As an Equal Opportunity and Affirmative Action Employer, UMMS recognizes the power of a diverse community and encourages applications from individuals with varied experiences, perspectives, and backgrounds.

The Friedrich Miescher Institute for Biomedical Research (FMI) invites applications for a tenure track group leader position (equivalent to an assistant professor) in its **Signaling & Cancer** program. We are seeking an outstanding individual who will establish an ambitious research program focused on fundamental questions in cancer biology. The successful applicant will have a demonstrated track record in the area of stem cell biology and/or molecular mechanisms of cancer development.



Friedrich Miescher Institute
for Biomedical Research

Group Leader Position, tenure track Stem Cell Biology and Cancer

Opportunities exist for collaborative interactions with the other FMI research programs of **Epigenetics** and **Neurobiology**. The FMI provides core facilities for experimental mouse genetics, high-end microscopy, cell sorting, genomics, protein crystallography, proteomics and bioinformatics. A highly competitive start-up package will be provided.

The FMI is an international biomedical research center with 300 members, including about 180 postdoctoral fellows and graduate students. It is part of the Novartis Research Foundation and is associated with the University of Basel. The Institute runs a successful international PhD program (for further information see www.fmi.ch). Situated in Basel, Switzerland, the FMI offers an outstanding english-speaking scientific and cultural environment in the center of Europe.

Applications, including a CV, the names and emails of three referees and a concise description of research interests and future plans should be submitted online at:

www.fmi.ch/gl_search

Informal inquiries can be sent to:
Dr. Brian A. Hemmings
(brian.hemmings@fmi.ch)

The closing date for applications is
December 31, 2010.

The Friedrich Miescher Institute for Biomedical Research (FMI) invites applications for a tenure track group leader position (equivalent to an assistant professorship). We are seeking an outstanding individual who will establish an ambitious research program focused on fundamental questions in the field of genomic stability. Candidates intending to carry out independent research in one of the following areas are particularly encouraged to apply: DNA repair, genomic replication, the replication-transcription interface, repeat instability, chromatin remodeling, telomeres or centromeres.



Friedrich Miescher Institute
for Biomedical Research

Group Leader Position, tenure track Genomic Stability and Cancer

The FMI is an international biomedical research center with 300 members, including about 180 postdoctoral fellows and graduate students. It is part of the Novartis Research Foundation and is associated with the University of Basel. The Institute runs a successful international PhD program (for further information see www.fmi.ch). Situated in Basel, Switzerland, the FMI offers an outstanding scientific and cultural environment in the center of Europe.

Opportunities exist for collaborative interactions with the other FMI research programs located within the focal areas of **Signaling & Cancer**, **Epigenetics** and **Neurobiology**.

The Institute provides core facilities for experimental mouse genetics, high-end microscopy, cell sorting, genomics, protein crystallography, proteomics and bioinformatics. A highly competitive start-up package will be provided.

Applications, including a CV, the names and emails of three referees and a concise description of research interests and future plans should be submitted online at:

www.fmi.ch/gl_search

Informal inquiries can be sent to:
Dr. Nico Thomä (nicolas.thomae@fmi.ch)
Dr. Susan Gasser (susan.gasser@fmi.ch)

The closing date for applications is
December 31, 2010.



The Institute of Molecular Biology gGmbH funded by the Boehringer Ingelheim Foundation

is looking for

Research Group Leaders (#100924a)

The **Institute of Molecular Biology (IMB)** Mainz, Germany, is a new research center of excellence situated on the campus of the University of Mainz, and integrated into its scientific community. IMB will open in Spring 2011. Research at IMB is generously funded by the Boehringer Ingelheim Foundation with 100 Million Euros already committed to research support. IMB research is interdisciplinary, focusing on epigenetics, RNA biology, DNA/RNA editing and DNA repair. Group leaders will investigate nuclear function and gene regulation, from the structural to the organismic level, and will span development, neurobiology, immunity and aging. We envision a collaborative mix of biologists, bioinformaticians and physicists covering a wide range of technologies, including Next-Generation Sequencing, advanced light microscopy, molecular cell biology, biochemistry and genetics, to computer simulations. Research is supported by strong core facilities.

Research of the new groups should focus on one or more of the following fields

- **Epigenetics**
- **DNA/RNA editing**
- **DNA repair**
- **RNA biology**

We seek outstanding, interactive candidates with an innovative research profile in the aforementioned fields and a strong record of publication. Preference will be given to candidates that integrate into their research an in vivo approach, notably development.

The positions are for 5 years initially, and can be renewed. The 5-year start-up packages for Group Leaders include an attractive salary and generous start-up funds for colleagues (postdoc, PhD student and technical) and supplies.

There are no teaching obligations, but teaching opportunities are available at the University of Mainz, e.g. for individuals wishing to obtain a German Habilitation.

Please apply online to (personal@imb-mainz.de) and include a cover letter, CV and a concise description of research interests & future research plans, quoting #100924a. Please also arrange for 3 letters of recommendation to (personal@imb-mainz.de).

Further information on IMB can be found at <http://www.imb-mainz.de> and questions can be addressed to the director (niehrs@dkfz.de). **Application deadline is December 3rd 2010.**

FACULTY POSITIONS



Penn Medicine

University of Pennsylvania School of Medicine seeks candidates for several Assistant or Associate Professor positions in the tenure track. Rank will be commensurate with experience. The successful applicant will have experience in the field of chromatin biology with a focus on epigenetics. Responsibilities include research addressing outstanding questions in chromatin biology and epigenetics, using genomic, genetic, structural, biochemical or systems approaches. It is anticipated that successful candidates will develop independent, interactive research groups and excel in interdisciplinary training. Applicants must have an M.D. or Ph.D. or M.D./Ph.D. degree and have demonstrated excellent qualifications in Research.

The Penn Epigenetics Program encompasses a broad range of research groups at the University of Pennsylvania and within the Philadelphia region. State-of-the-art laboratory space, including shared computational and experimental core facilities, will be available in the Fisher Translational Research Building, to be completed in the fall of 2010.

The University of Pennsylvania is an equal opportunity, affirmative action employer. Women and minority candidates are strongly encouraged to apply.

Apply for this position online at:

http://www.med.upenn.edu/apps/faculty_ad/index.php/g305/d2104

UC DAVIS

Inorganic Materials Chemistry Faculty Position in the Energy for the Future Initiative at the University of California, Davis

The UC Davis Department of Chemistry invites applications for an Assistant Professor of Chemistry associated with the UC Davis Energy for the Future Initiative targeting major energy issues facing California and the nation. The Energy for the Future Initiative brings a total of fourteen new faculty positions to the campus, including a total of four positions in the Chemistry Department. The online application link is available on the Chemistry website (<http://www.chem.ucdavis.edu/>).

We seek a colleague who will synthesize new inorganic solid state compounds, characterize their structures, properties, and/or stability, with the fundamental goal of understanding the inorganic solid state at the level of atoms and chemical bonds, and the practical goal of finding new materials for energy applications. He/she will develop an independent research program and also take advantage of specialized techniques and major instrumentation, including the Peter A. Rock Thermochemistry Laboratory, TEM and spectroscopy at UC Davis, as well as synchrotron and neutron based experimentation. A competitive candidate will demonstrate an interest in actively participating in the UC Davis Energy Institute, as well as demonstrating a strong commitment to undergraduate and graduate teaching. A Ph.D. or equivalent degree in chemistry or a related discipline is required. The position is open until filled. To ensure full consideration, online applications should be submitted no later than **November 1, 2010**, for a targeted start date of **July 1, 2011**.

*The University of California is an
Affirmative Action/Equal Opportunity Employer.*

CAREERS IN BIOENGINEERING AND NANOTECHNOLOGY

The Institute of Bioengineering and Nanotechnology in Singapore is seeking highly motivated individuals who are interested in making an impact in advancing research and development in the following areas:

Drug and Gene Delivery

where the controlled release of therapeutics involve the use of functionalized polymers, hydrogels and biologics for targeting diseased cells and organs, and for responding to specific biological stimuli.

Cell and Tissue Engineering

where biomimicking materials, stem cell technology, microfluidic systems and bioimaging tools are combined to develop novel approaches to regenerative medicine and artificial organs.

Biosensors and Biodevices

which involve nanotechnology and microfabricated platforms for high-throughput biomarker and drug screening, automated biologics synthesis, and rapid disease diagnosis.

Pharmaceuticals Synthesis and Green Chemistry

which encompasses the efficient catalytic synthesis of chiral pharmaceuticals, and new nanocomposite materials for sustainable technology and alternative energy generation.



Positions are available for **Senior Group Leader, Group Leader, Principal Research Scientist, Senior Research Scientist, Research Scientist, Postdoctoral Fellow, Research Officer and Lab Officer** in IBN's four research areas.

We provide competitive salaries as well as attractive benefits. Remuneration will commensurate with qualification and experience.

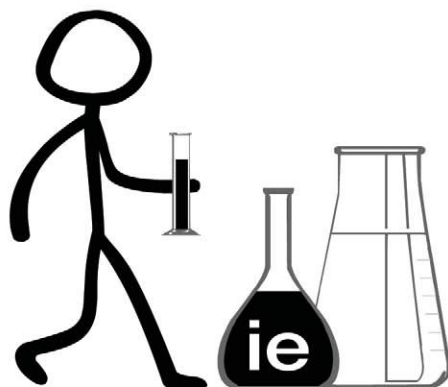
If you are interested in joining a multi-disciplinary research institute at the cutting edge of bioengineering and nanotechnology, please forward a cover letter, your curriculum vitae, and a list of three references to:

Prof. Jackie Y. Ying, Executive Director
Institute of Bioengineering and Nanotechnology
31 Biopolis Way, The Nanos, #04-01, Singapore 138669
Email: recruit@ibn.a-star.edu.sg
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POSITIONS OPEN

ASSOCIATE OR FULL PROFESSOR Biology (Anticipated Opening)

Job ID: 3300 | Location: Hunter College

GENERAL DUTIES Performs teaching, research, and guidance duties in area(s) of expertise as noted below. Shares responsibility for committee and department assignments, performing administrative, supervisory, and other functions as may be assigned.

CAMPUS SPECIFIC INFORMATION Successful candidates will be expected to have and sustain active, independent programs of extramurally funded research in Molecular and Cellular Biology to build upon and complement existing strengths in the Department of Biological Sciences. Teaching in one or more of these areas at the undergraduate and graduate levels is expected.

QUALIFICATIONS Ph.D. in Molecular Biology, Cell Biology, or a related area. The candidate must have a demonstrated commitment to research and teaching, outstanding publications in peer-reviewed journals, and a record of extramural funding and team management experience.

COMPENSATION Salaries and start-up packages are competitive, commensurate with experience.

HOW TO APPLY Please send curriculum vitae, a statement of research interests, representative publications, and names of references by electronic submission to the following e-mail address: biofac@hunter.cuny.edu

CLOSING DATE The search will remain open until the position is filled.

EQUAL EMPLOYMENT OPPORTUNITY The City University of New York is an Equal Opportunity Employer which complies with all applicable laws and regulations, and encourages inclusive excellence in its employment practices.

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UNIVERSITY of MISSOURI



Department of Surgery Research Faculty

The University of Missouri Department of Surgery is seeking qualified candidates to join the Division of Acute Care Surgery as a tenure-track faculty researcher dedicated to full-time translational research in critical care. Ideal candidates will have a Ph.D. or M.D./Ph.D., have a demonstrable track record in critical care research, and a proven ability to obtain extramural funding for research endeavors. Academic rank will be contingent upon experience and history of publication.

New faculty members will become a part of the accredited surgical and critical care training program. Participation in research which further enhances the educational offerings to residents and fellows and promotes collaboration with other faculty in the Division is expected.

The position offers an outstanding salary and benefits package, paid relocation, and a research support package commensurate to rank, title, and area of research. Applications from minorities and women are encouraged.

The University of Missouri is located in the heart of the state, halfway between St. Louis and Kansas City, and is the flagship campus for the University of Missouri system. Columbia boasts affordable housing, a cost of living below the national average, diverse cultural and economic opportunities, varied recreational facilities, quality health care, and excellent public schools making it one of the most desirable places to live in the United States. Most people who choose to live in this college town of just under 100,000 population consider themselves as having the best of both worlds---small-town convenience and warmth with big-city sophistication and amenities. For more information about the Columbia community, visit www.gocolumbiamo.com.

Applicants should send curriculum vitae to: Assistant to the Chairman, Department of Surgery, University of Missouri-Columbia, Health Sciences Center, 1 Hospital Drive DC077.00, Columbia, Missouri, 65212.

The University of Missouri is an Affirmative Action/Equal Opportunity Employer and complies with ADA Act of 1990. Women and minorities are encouraged to apply

Visit the University of Missouri-Columbia's web site at <http://mujobs.missouri.edu>



**MIAMI
UNIVERSITY**

**Director of the Institute for the
Environment and Sustainability**

Tenured Full or Associate Professor, to provide leadership for the programs of the Institute, coordinate among the Institute and other academic units and centers of the University, and enhance our national profile of excellence in the environment and sustainability.

The Institute was founded in 1970 as the Institute of Environmental Science and its master's program has produced over 600 alumni who are today working in the commercial, governmental, and non-profit sectors to make our environments healthier and natural resource use more sustainable. It is being restructured to include a PhD program in Ecology, Evolution, and Environmental Biology and two undergraduate co-majors focusing on environmental science and sustainability. It includes 4 staff and more than 45 faculty drawn from 13 departments and 4 divisions of the University.

The Director will coordinate with chairs and directors of other academic units in furthering the work of the Institute, pursue external funding, maintain active scholarship, teach appropriate courses, and supervise the Institute staff. Requirements include a doctorate in an area of environmental science, engineering, policy, or related field; a strong record of scholarship; success in extramurally funded research; and professional experience with applied environmental and sustainability issues. Teaching experience and demonstrated success in administrative duties desired.

Please send letter of nominations or application, curriculum vitae, statement of research and teaching interests, and contact information for three referees to **Dr. Bill Renwick, Search Committee Chair, Department of Geography, 216 Shideler Hall, Miami University, Oxford, OH 45056.** For more information, phone 513-529-5811 or 513-529-5010 or e-mail renwicwh@muohio.edu. Review of applications will begin **December 1, 2010**. Position available August 2011. For more information about the University and the Institute see <http://www.miami.muohio.edu/> and <http://www.cas.muohio.edu/ies/>. For information regarding campus crime and safety, visit www.muohio.edu/righttoknow.

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**Faculty Position
Structural Biology Program
Sloan-Kettering Institute**

The Structural Biology Program of the Sloan-Kettering Institute (www.ski.edu) invites applications for a tenure-track faculty position at the Assistant Member level (equivalent to Assistant Professor). We are interested in outstanding individuals who have demonstrated records of significant accomplishment. Areas of interest include x-ray crystallography, NMR spectroscopy, EM and optical imaging, as well as the interface of structural, chemical and computational biology. Faculty will be eligible to hold graduate school appointments in the Gerstner Sloan-Kettering Graduate School of Biomedical Sciences, the Weill Cornell Graduate School of Medical Sciences, as well as the Tri-Institutional MD/PhD Training Program.

The deadline for applications is **November 1, 2010**. Interested candidates should visit <http://facultysearch.ski.edu> to apply via the on-line faculty application. Informal inquiries may be sent to **Julie Kwan** at kwanj@mskcc.org or to **Dr. Nikola Pavletich, Chair, Structural Biology Program** at pavletin@mskcc.org. MSKCC is an equal opportunity and affirmative action employer committed to diversity and inclusion in all aspects of recruiting and employment. All qualified individuals are encouraged to apply.



**Memorial Sloan-Kettering
Cancer Center**
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**Herchel Smith Postdoctoral
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(Six Posts)**

Academic Division

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Biological Sciences and Physical Sciences

The Managers of the Herchel Smith Postdoctoral Research Fellowships Fund invite applications within the fields of Biological Sciences, Climate Research, Materials for Sustainable Energy and Pure Mathematics.

All Fellowships are to be held at the University of Cambridge from 1 October 2011 or otherwise by negotiation. Fellowships are available for between two and three years and provide an opportunity for independent research, although the holders will usually work in close collaboration with established research groups.

In accordance with Dr Smith's will, candidature is limited to candidates who have completed their PhD degree, or equivalent, within the last three years at any university but normally excluding Cambridge and Harvard.

The stipend will be on the University's Postdoctoral Research Associate scale, currently £27,319 to £35,646 per annum with £10,000 per annum as research allowance.

Further details are available at www.herschelsmith.cam.ac.uk/fellowships or from the Secretary to the Fund Managers, telephone: +44(0)1223 761507 or e-mail hshf@admin.cam.ac.uk Please quote reference: AK07236. Closing date: 26 November 2010.

The University is committed to Equality of Opportunity.



**The University of Texas
at Austin**

**Cancer Molecular Biology Position
The Institute for Cellular and Molecular Biology**

The Institute for Cellular and Molecular Biology, Alan Lambowitz, Director, invites applications for a tenure-track/tenured position in cancer molecular biology. Academic appointments at the level of Assistant, Associate, or Full Professor will be in the section of Molecular Genetics and Microbiology in the College of Natural Sciences. Candidates should have an outstanding record of research productivity and a research plan that utilizes molecular and biochemical approaches to address important problems in cancer biology. Areas of particular interest include but are not limited to DNA damage responses, genome instability, post-translational regulatory mechanisms, chromatin, and small regulatory RNAs.

Building on a strong existing faculty, the Institute has recruited more than 50 new faculty members over the past ten years. In addition to its highly interactive and interdisciplinary research environment, the Institute provides administrative and financial support for the Graduate Programs in Cell and Molecular Biology, Microbiology, and Biochemistry, and state-of-the-art core facilities including DNA sequencing, mass spectrometry, electron and confocal microscopy, DNA microarrays, robotics, mouse genetic engineering and Next-Gen sequencing. An MD-PhD program with the UT Medical Branch and the new Dell Pediatrics Research Institute enhance the environment for basic Biomedical Research.

Austin is located in the Texas hill country and is widely recognized as one of America's most beautiful and livable cities.

Please send a single PDF file containing your curriculum vitae, summary of research interests and names of three references before November 1, 2010 to: mgm_search@biosci.utexas.edu. References may also send their letters directly to the same email address.

Homepages • <http://www.icmb.utexas.edu> • <http://www.biosci.utexas.edu/MGM> •

*The University of Texas at Austin is an Equal Opportunity Employer.
Qualified women and minorities are encouraged to apply; a background
check will be conducted on applicant selected.*

Associate or Full Professor with Tenure – Department of Symptom Research

The University of Texas MD Anderson Cancer Center
Houston, Texas, United States

The Department of Symptom Research at MD Anderson is seeking an established clinical or basic scientist (M.D. or Ph.D.) to contribute to a major effort to examine the mechanisms of cancer symptom expression and develop novel methods of symptom management for such symptoms as pain, fatigue, emotional distress, disrupted sleep, neuropathy, and other symptoms that limit treatment tolerability and impair survivorship. The approach is multidisciplinary, including biological, behavioral, and clinical scientists. The applicant must be willing to develop projects within the context of this multidisciplinary approach to research. Active program includes animal and human studies, symptom assessment methods, and randomized clinical trials with symptom reduction or prevention as primary outcomes. Current studies include various cancers and cancer treatments. The program is well funded with program project and other National Institutes of Health, private foundation, and pharmaceutical industry grants.

We expect candidates with a well-established record of funded research and significant research accomplishment, both of which are required for a tenured faculty appointment in a major academic research institution. This position is available immediately.

Applications are currently being reviewed. To inquire about the position or to submit an application, please respond by e-mail to **Nyma A. Shah, CCRP**, Department Administrator at nashagh@mdanderson.org



MD Anderson Cancer Center is an equal opportunity employer and does not discriminate on the basis of race, color, national origin, gender, sexual orientation, age, religion, disability or veteran status, except where such distinction is required by law. All positions at MD Anderson are considered security sensitive; drug screening and thorough background checks will be conducted. The University of Texas MD Anderson Cancer Center values diversity in its broadest sense. MD Anderson is a smoke-free environment.



DIRECTOR International Institute for Applied Systems Analysis

The International Institute for Applied Systems Analysis (IIASA, www.iiasa.ac.at), located near Vienna, Austria, is seeking a highly qualified scientific leader for the position of Director beginning on 1 January 2012. The successful candidate will be responsible for overall management and direction of the Institute, guiding a diverse research program that combines natural and social science with systems analysis approaches to identify and assess possible solutions to complex global problems. Candidates should combine a vision for IIASA with scientific excellence, management and diplomatic skills, fundraising accomplishments, and broad experience in inter-disciplinary, international research and policy applications. The Director should be an effective and active advocate to expand participation and membership in IIASA and enhance its capacity building activities. The Director supervises approximately 200 scientists and support staff from 30 countries.

IIASA is nongovernmental, sponsored by an international consortium of 17 National Member Organizations. Applicants should have excellent written and spoken English, IIASA's working language. The Institute's management and staff alike are committed to a working environment that promotes equality, diversity, and tolerance.

The post is a 3-year position with the possibility of renewal. Salary and benefits are competitive with comparable international organizations. Review of applications will begin on **1 January 2011**. Submit letter of application, a statement on the future development of policy-relevant interdisciplinary research as it pertains to IIASA, CV including a description of scientific and professional achievements, bibliography, and contact information for three references, to:

Professor Peter Lemke, Chairman of the Search Committee
Alfred-Wegener-Institut für Polar- und Meeresforschung
Postfach 12 01 61, D-27515 Bremerhaven, Germany
Phone: (+49-471) 4831-1751; Email: peter.lemke@awi.de

DIRECTOR SOUTHWEST NATIONAL PRIMATE RESEARCH CENTER

The Southwest National Primate Research Center (SNPRC) located at the Southwest Foundation for Biomedical Research (SFBR) invites applications and nominations for the position of Director. The SNPRC serves investigators around the globe, providing specialized technical capabilities and unique research animals for studies with nonhuman primates. The strengths of the SNPRC include the world's largest captive baboon population, the world's largest and best characterized pedigreed primate population, the largest chimpanzee census of any NPRC, a well characterized marmoset colony, and a SPF rhesus macaque colony. The Southwest Foundation has the world's largest group of geneticists committed to research with, and management of, nonhuman primates, outstanding expertise in nonhuman primate virology and immunology, extensive technical capabilities for supporting research with a broad range of nonhuman primates, and ABSL-3 and ABSL-4 biocontainment with select agent capabilities.

The SNPRC Director provides overall leadership for the SNPRC which currently has a total of 174 staff members distributed across four units: Research Resources, Veterinary Resources, Translational Science, and Financial Administration. The Center Director also is responsible for the day to day management and scientific direction of the SNPRC. The administrative staff of the Primate Center includes an Associate Director for Research Resources, an Associate Director for Veterinary Resources, an Assistant Director for Veterinary Resources, and an Assistant Director for Scientific Administration. The new Director will report directly to the SFBR President and CEO, and will work closely with the Chief Scientific Officer of SFBR to advance the joint missions of SFBR and the SNPRC.

The Director will be an innovative and thoughtful leader and an accomplished scientist with extensive administrative experience in a research setting. The successful candidate will have a history of building research programs, a strong record of NIH grant funding, experience in nonhuman primate research, and outstanding writing skills. As the primary spokesperson for the SNPRC animal research programs, the successful candidate must also have excellent communication skills and be a powerful advocate for nonhuman primate research.

Qualified applicants must have a doctoral degree, (e.g., Ph.D., D.V.M., M.D., etc.) and sufficient relevant professional experience in research or administration at a major university or non-profit research institution to be appointed at the rank of Scientist (equivalent to Full Professor in a university environment). The final appointment of the new SNPRC Director must be approved by the National Center for Research Resources of the NIH.

Located in San Antonio, Texas, one of the fastest growing biomedical centers in the country, SFBR offers attractive salary and benefits packages. Nominations and applications should be sent to the **Chair, SNPRC Director Search Committee, c/o Human Resources Office, Southwest Foundation for Biomedical Research, P.O. Box 760549, San Antonio, Texas 78245-0549**. Nominations and applications should include a letter outlining qualifications and fit to the position. In addition, applications should include a CV and the names and contact information for at least three references. Review of applications will begin on **November 5, 2010**.

Additional information about the SNPRC can be found at www.snprc.org. Additional information about SFBR can be found at www.sfbr.org. Go to www.sfbr.org/pages/employmentposting.php for additional information about this position and application procedures.



FACULTY POSITION RIKEN BRAIN SCIENCE INSTITUTE

The Brain Science Institute (BSI) of RIKEN (Institute of Physical and Chemical Research) in Wako, Japan, is seeking outstanding neuroscientists for a tenure-track Team Leader position (equivalent to a U.S. Assistant or Associate Professor).

The area and theme of research we wish to strengthen are broad, covering molecular, cellular, genetic, neural circuit and systems neuroscience in health and disease. We are particularly interested in investigators who study basic mechanisms of nervous system function using an interdisciplinary approach that includes some combination of genetics, electrophysiology, optical imaging and behavioral neuroscience.

Candidates should have demonstrated the strong ability to develop a significant and original research program.

A generous annual research fund and startup package will be provided, in addition to an apartment/condo rental allowance and other employee benefits.

The Search Committee will begin reviewing applications immediately. The search will continue until the position is filled. Applicants should submit a curriculum vitae, a summary of current and proposed research, and arrange for three letters of recommendation, all to be sent to:

Search Committee 1, RIKEN Brain Science Institute
2-1 Hirosawa, Wako, Saitama 351-0198, Japan
Fax: +81-48-467-9683; Email: search20101@brain.riken.jp
<http://www.brain.riken.jp>

RIKEN-BSI is an Equal Employment Opportunity Employer. Women are strongly encouraged to apply.

UNIVERSITY OF CANTABRIA (SPAIN) - INTERNATIONAL CAMPUS OF EXCELLENCE



OPEN POSITIONS FOR SENIOR RESEARCHERS IN STRATEGIC AREAS:

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Deadline, 31st October 2010

Structural Biologist/Biophysicist Biomolecular Structure and Function

The Department of Biochemistry and Molecular Biology at The University of Texas MD Anderson Cancer Center is seeking an outstanding scientist with a strong research background in biomolecular structure and function. The Department has an opening for one full-time, tenure track faculty member at the level of assistant/associate professor. Academic rank and salary shall be commensurate with experience and qualifications. The applicant should have demonstrated excellence in biological problem-driven research that complements Departmental strengths in developmental biology, chromatin/epigenetics, signal transduction and cancer biology. Although not required, one or more of following areas of expertise will be preferred: X-ray crystallography and/or other structural biology methods, single molecule methods, biophysical assay of biomolecular interactions. Successful applicants must have a doctoral degree, post-doctoral experience, and are expected to demonstrate scholarly activities, to establish their own independent research program, and must be competitive in securing federal and non-federal grant support. The candidate will also be expected to participate in graduate student education.

Interested applicants should send a cover letter, a CV, a brief research proposal and three letters of reference to:

D. Watkins, Coordinator Faculty Search
Department of Biochemistry and Molecular Biology, MD Anderson
Cancer Center, Unit 1000, 1515 Holcombe Boulevard, Houston, Texas
77030, USA; SBBS@mdanderson.org

Receipt deadline: **15th November 2010.**



MD Anderson Cancer Center is an equal opportunity employer and does not discriminate on the basis of race, color, national origin, gender, sexual orientation, age, religion, disability or veteran status, except where such distinction is required by law. All positions at MD Anderson are considered security sensitive; drug screening and thorough background checks will be conducted. The University of Texas MD Anderson Cancer Center values diversity in its broadest sense. MD Anderson is a smoke-free environment.

FACULTY POSITION IN VIROLOGY

The Department of Microbiology & Immunology at SUNY Upstate Medical University in Syracuse, NY, invites applications for a tenure-track faculty position at the Assistant/Associate Professor level. Applicants must have a PhD, MD, and/or DVM degree (or equivalent), postdoctoral experience, and a strong publication record. Successful candidates will be expected to develop or bring an independent research program in virology. Areas of interest include, but are not limited to, viral pathogenesis, virus-host interactions, animal models of virus diseases, immune responses to viruses, oncogenic viruses, and translational research in antiviral therapy and prevention.

SUNY Upstate Medical University is a thriving biomedical research enterprise and the region's only academic medical center. The university has significant core facilities, including a state-of-the-art SCID mouse facility, flow cytometry instruments capable of sorting cells under BSL-2 conditions, mass spectrometry and proteomics, microarray, and DNA sequencing. An excellent start-up package, modern laboratory space, and state-funded salary commensurate with experience will be provided. All department faculty are expected to participate in teaching graduate and medical students.

Candidates should apply by sending a cover letter, CV, and a summary of current and future research interests to microimm@upstate.edu or to:

Virology Search Committee, c/o Michelle Lonkey
SUNY Upstate Medical University
750 E. Adams Street, 2204 Weiskotten Hall
Syracuse, NY 13210

Send letters of recommendation only upon request from the committee.

Review of applications will begin on November 1st and will continue until the position is filled. For additional information, visit the department website www.upstate.edu/microb, or email Dr. Jennifer Moffat, chair of the search committee at microimm@upstate.edu.



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**THE UNIVERSITY
OF IOWA**

Faculty Positions in the Department of Biology

The Department of Biology at the University of Iowa is notable for its multidisciplinary diversity, with strengths in Evolution, Genetics, Developmental/Cell Biology, and Neurobiology. Information about the Department and the Roy J. Carver Center for Genomics is available at www.biology.uiowa.edu. The Department of Biology is located in recently renovated space and provides competitive salaries and benefits along with strong infrastructure support for research. Applications are invited for two faculty positions.

Evolutionary Genomics: We are seeking candidates at the senior level who utilize high throughput experimental, bioinformatics and/or theoretical-modeling approaches to address fundamental questions in genome and/or gene expression evolution. Candidates for this position are expected to be tenured Associate or Full Professors and to play a leadership role in the Department of Biology. Areas of particular interest include but are not limited to: comparative genomics and transcriptomics, phylogenomics, transcriptional regulatory evolution and networks, genetic basis of gene expression variation, and computational approaches to systems biology. Individuals using established or emerging model genetic systems are particularly encouraged to apply. The successful candidate must have an excellent track record of extramural funding and an international reputation in the candidate's area of research as well as an interest in teaching advanced Genomics/Bioinformatics courses.

Evolutionary Ecology: We are seeking candidates at the Assistant/Associate Professor level who are addressing questions at the interface of ecology and evolution using field, laboratory and/or theoretical modeling approaches. Areas of particular interest include but are not limited to: metapopulation dynamics, interspecific interactions, species invasiveness, constraints on species range limits, environmental genomics, and adaptation of natural populations. Recognition in the candidate's area of research is desirable. The successful candidate will be expected to contribute to teaching advanced courses relevant to the interdisciplinary program in Environmental Sciences (www.uiowa.edu/~envsci).

Successful candidates must have a PhD in one of the biological sciences, postdoctoral experience, a recognized record of accomplishment as reflected in publications in leading journals, the ability to establish and maintain an extramurally funded research program, and an interest in participating in the department's teaching mission. Desirable qualifications include the potential for productive interactions with faculty in the Department, and other life scientists at the University of Iowa, and expertise in emerging technologies and methodologies. Applications should be submitted online at <http://jobs.uiowa.edu> under requisition 58449 for the **Evolutionary Genomics** position and requisition 58450 for the **Evolutionary Ecology** position. Applicants should submit a cover letter, *curriculum vitae*, a statement of research objectives and teaching interests, at most 4 publications and the names of 3 references. Formal screening of applications will begin **November 15, 2010** and continue until the positions are filled.

The Department of Biology and the College of Liberal Arts and Sciences are strongly committed to gender and ethnic diversity; the strategic plans of the University and College reflect this commitment. Women and minorities are encouraged to apply. The University of Iowa is an Affirmative Action/Equal Opportunity Employer.

MONTEREY BAY AQUARIUM RESEARCH INSTITUTE

2011 POSTDOCTORAL FELLOWSHIP PROGRAM

Founded in 1987 and supported by the David and Lucile Packard Foundation, The Monterey Bay Aquarium Research Institute (MBARI) is a non-profit oceanographic research institute, dedicated to the development of state-of-the-art instrumentation, systems, and methods for scientific research in the oceans. MBARI's research center includes science and engineering laboratories, as well as an operations facility to support our research vessels and oceanographic equipment, including remotely operated and autonomous underwater vehicles. Located in Moss Landing, California, the heart of the nation's largest marine sanctuary, MBARI places a balanced emphasis on science and engineering, with established programs in marine robotics, ocean physics, chemistry, geology, and biology, as well as information management and ocean instrumentation research and development.

MBARI invites applications each year for several postdoctoral fellowships in the fields of biological, chemical, and physical oceanography, marine geology, and ocean engineering. Fellowships may require occasional trips to sea. Awards are typically for two years.

Candidates must be awarded their Ph.D. degree prior to commencing the 2 year appointment between September 2011 and March 2012.

Applicants are encouraged to communicate with potential research sponsors at MBARI for guidance on project feasibility, relevance to ongoing MBARI research, and resource availability (http://www.mbari.org/about/postdoc_mentors.htm).

Application deadline: Friday, December 10, 2010

Selected candidates will be contacted in early March 2011.

Application requirements:

1. Curriculum vitae
2. At least three professional letters of recommendation
3. Succinct statement of the applicant's doctoral research
4. Potential research goals at MBARI
5. Supplemental Information online form (http://www.mbari.org/oed/jobs/forms/postdoc_form_2011.htm)

Competitive compensation and benefits package.

MBARI considers all applicants for employment without regard to race, color, religion, sex, national origin, disability, or veteran status.

Address your application materials to:

MBARI, Human Resources
Job code: Postdocs-2011
7700 Sandholdt Road, Moss Landing, CA 95039-9644

Submit by e-mail to jobs_postdocs@mbari.org (preferred),
by mail, or fax to (831) 775-1620.



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**Director, Division of Biomedical Technology
National Center for Research Resources
National Institutes of Health
Department of Health and Human Services**



THE POSITION: The National Center for Research Resources (NCRR) is seeking exceptional candidates for the position of Director, Division of Biomedical Technology. This individual will lead a program that supports research to discover, create, and develop innovative technologies and provides access to these technologies to the biomedical research community. This research is conducted through grants for technology-driven research and development, the biomedical informatics research network, and biomedical technology research centers. The incumbent will supervise a staff of eleven, including nine scientific professionals in overseeing 50 Biomedical Technology Research Center Grants in five broad technology areas: Imaging, Informatics, Optical and Laser Technology, Technology for Structural Biology and Technology for Systems Biology. At these research centers, interdisciplinary teams create unique, transformative technologies and work to promote their widespread use. These innovations are accomplished through a synergistic interaction of technical and biomedical expertise, both within the centers and through intensive collaborations with other leading laboratories. These research centers span basic, translational and clinical research to create tools that advance science at the molecular level and change scientific approaches to diagnosis and treatment of disease. Additionally, the Division administers the shared instrumentation program which provides institutions with the funds to purchase state of the art technologies, helping researchers remain at the forefront of the biology and medicine. Lastly, the Division supports biomedical researchers through investigator-initiated research projects or large collaborative research projects with other NIH organizations and/or Federal agencies.

The NCRR provides laboratory scientists and clinical researchers with the environments and tools they need to understand, detect, treat, and prevent a wide range of diseases www.ncrr.nih.gov. This support enables discoveries that begin at a molecular and cellular level, move to animal-based studies, and then are translated to patient-oriented clinical research, resulting in cures and treatments for both common and rare diseases. This position offers a unique and exciting opportunity for an extremely capable individual to share responsibility in providing strong and visionary leadership to an organization dedicated to enhancing our understanding of health and disease, translating basic research into medical care, and improving human health.

QUALIFICATIONS REQUIRED: Applicants must possess a Ph.D., or equivalent degree, as well as senior-level research experience or knowledge of research programs moving research from the basic laboratory sciences into clinical research. Candidates should be outstanding communicators and known and respected as distinguished individuals of outstanding competence. Applicants should also demonstrate the ability to think strategically, work collaboratively, and use a consultative approach to problem solving and decision making.

SALARY/BENEFITS/OTHER INFORMATION: Salary is commensurate with experience and a full package of Civil Service benefits is available, including: retirement, health and life insurance, long term care insurance, leave and savings plan (401K equivalent). The National Institutes of Health inspires public confidence in science by maintaining high ethical principles. In addition to the Federal government's code of ethics, we have our own agency specific standards which are described at the NIH Ethics web site. <http://ethics.od.nih.gov/overview.htm> This position is subject to a background investigation.

HOW TO APPLY: A Curriculum Vitae, Bibliography, and two letters of recommendation must be received by **October 15, 2010**. Application packages should be sent to the **National Institutes of Health, National Center for Research Resources, ATTN: Sabrina Posley, 6701 Democracy Boulevard, Suite 206, Bethesda, Maryland 20892**.

For further information, please call **(301) 435-0717**. All information provided by candidates will remain confidential and will not be released outside the NCRR search process without a signed release from candidates.



Massachusetts
Institute of
Technology

Come work with us!

Faculty Position At MIT, The McGovern Institute For Brain Research

The McGovern Institute for Brain Research at MIT is seeking one faculty member at the Assistant Professor level. The Institute's focus is systems neuroscience with emphasis on the neural basis of perception, cognition, and action. We are seeking a candidate with a research focus in any of these three areas, using either human subjects or animal models. We would regard it as a plus if the candidate's work bridges levels using a variety of tools and/or if the candidate were interested in translating basic research findings into new ideas for studying the pathophysiology or treatment of brain disorders.

The mission of the McGovern Institute is to understand the relationship of neuronal processes, circuits and computations to behavior, ultimately providing benefits to human health and welfare. Research in the McGovern Institute is expected to help people with brain disorders ranging from sensory system impairments to movement disorders and emotional and cognitive disorders. McGovern Institute scientists have many opportunities for collaboration and are appointed through an MIT department and will have teaching responsibilities for their home department.

Applicants should submit a curriculum vitae, a summary of current and proposed research programs, a publication list, and should arrange for three letters of recommendation to be sent electronically via Academic Jobs Online (<https://academicjobsonline.org/ajol>). Please indicate which research model you are using, animal or human. Consideration of applications will begin on October 15, 2010. For more information on the McGovern Institute please visit our website at <http://mcgovern.mit.edu>



McGOVERN INSTITUTE
FOR BRAIN RESEARCH AT MIT

MIT is an Affirmative Action/Equal Opportunity Employer. Qualified women and minority candidates are especially encouraged to apply.

<http://web.mit.edu>



University of Iowa Health Care

DIRECTOR, Transgenic and Genome Manipulation Core Research Facility

The University of Iowa Carver College of Medicine is seeking a Director for a newly consolidated core research support facility in genetic manipulation of the mammalian genome. This includes generation of transgenic mice by pronuclear injection, manipulation of embryonic stem cells for the generation of knockout and knockin mice, and development and use of inducible pluripotent stem cells. Level of appointment will be Associate Research Scientist or Research Scientist with commensurate salary based on experience and qualifications.

For more information and to apply for this position, go to <http://jobs.uiowa.edu/> and click on requisition #58493.

Women and minorities are strongly encouraged to apply. The University of Iowa is an Equal Opportunity/Affirmative Action Employer.



Tufts
UNIVERSITY

TENURE TRACK FACULTY POSITION Animal Physiology

The Department of Biology at Tufts University invites applications for a tenure-track Assistant Professor in Animal Physiology to begin in September, 2011. We seek a creative scholar whose research program focuses on elucidating the mechanisms by which animals (vertebrates or invertebrates) interact with, or adapt to, their environment. Possible research areas include neuroethology, endocrinology, biomechanics and physiological ecology. Applicants should employ an integrative approach that addresses how systems function across multiple levels of biological organization.

The successful candidate is expected to develop an active externally funded research program involving graduate and undergraduate students. A clear commitment to teaching excellence at the undergraduate and graduate levels is essential. Doctoral degree and a record of research productivity are required. Postdoctoral experience preferred.

Applicants should submit the following in a single PDF file: (1) a cover letter, (2) curriculum vitae, (3) statement of research interests and plans and (4) statement of teaching experience and philosophy. Submission of 1-3 selected reprint PDFs is encouraged, but not required. Applications and three letters of reference should be sent to karin.murphy@tufts.edu.

Review of applications begins **November 30, 2010**, and continues until the position is filled.

Tufts University is an Affirmative Action/Equal Opportunity employer. We are committed to increasing the diversity of our faculty. Members of underrepresented groups are strongly encouraged to apply.

UNIVERSITY OF MINNESOTA, Twin Cities Department of Genetics, Cell Biology and Development Tenure-track Faculty Position in Cellular Dynamics and Imaging

The Department of Genetics, Cell Biology and Development is recruiting candidates for a tenure-track position as the Assistant Professor level. We seek investigators who are addressing fundamental questions concerning intracellular transport mechanisms using state of the art imaging and quantitative approaches, including computational methods. The department is recognized for its expertise in the structure/function of the cytoskeleton, the developmental genetics of model organisms (including worms, flies and mice), transposon biology and genome manipulation, human and cancer genetics, and chromosome instability. We have recently recruited faculty members with interests in cell cycle control, chromosomal segregation, protein degradation, membrane trafficking, cell adhesion, and signaling. Competitive candidates will complement and extend existing departmental strengths, while building links to related translational research efforts at the University in the areas related to treatment of diabetes, cancer, cardiovascular disease and neurodegeneration.

Candidates must have a Ph.D. and/or M.D. degree, at least three years of post-doctoral experience, a strong publication record, and they must also be U.S. citizens or be able to secure permanent resident status. Successful candidates will be expected to develop independent, externally funded research programs, interact collaboratively among a variety of disciplines, and participate in the undergraduate, graduate, and/or professional teaching programs of the Department. Starting salaries will be commensurate with education and experience, and competitive startup packages are available. For additional information on the Department see <http://www.gcd.umn.edu/>.

Individuals interested in this position should visit (<https://employment.umn.edu>) and search for requisition #168470. Follow instructions to Upload/Attach a CV and statement of research interests. In addition, three letters of recommendation that consider both research and teaching potential should be sent electronically as PDF files to: **Mary Muwahid** (e-mail: muwahid001@umn.edu) Review of applications will begin **November 10, 2010** and will continue until the position is filled.

The University of Minnesota shall provide equal access to and opportunity in its programs, facilities, and employment without regard to race, color, creed, religion, national origin, gender, age, marital status, disability, public assistance status, veteran status, sexual orientation, gender identity, or gender expression.



2 Faculty Positions in Estuarine and Marine Processes (rank open)

University of Maryland Center for Environmental Science Chesapeake Biological Laboratory

We invite applications for two tenure-track faculty positions in marine biogeochemistry and marine/estuarine ecology that will complement and enhance our existing programs in biogeochemistry, ecology, fisheries science and ecotoxicology. Applications are particularly encouraged from:

Organic biogeochemist – including the biogeochemistry of biologically important elements, or emerging contaminant pathways and biomagnification in food webs. Expertise in new, rapidly expanding fields e.g., ocean acidification, geomicrobiology, or nano-scale biogeochemistry, is particularly encouraged. A strong analytical focus is desirable.

Marine/estuarine ecologist – including processes related to fluxes through lower trophic levels and benthic environments, ecosystem responses to perturbations, and a cross disciplinary systems approach to evaluating ecosystem structure and function. Candidates whose research integrates field work and modeling are encouraged.

Full details are available at www.umces.edu/cbl/employment. Successful applicants will develop a rigorous, high-profile, extramurally funded research programs. Faculty members are expected to teach one graduate course every other year. There is no undergraduate teaching. Salary and benefits are competitive and dependent on qualifications. Ph.D. is required of successful candidates at time of appointment and postdoctoral experience is preferred. For full consideration, please send a statement of interest that summarizes your research and interests, a c.v., examples of relevant publications and a list of 4 potential references to **Ms. Kelly Arthur** (arthur@cbl.umces.edu) with either Biogeochemist or Marine Ecologist in the subject line by **December 20, 2010**.

*UMCES is an Affirmative Action/Equal Opportunity Employer.
We promote excellence through diversity and encourage women and minorities to apply.*



CHAIR Pharmacological and Pharmaceutical Sciences University of Houston College of Pharmacy

The College of Pharmacy invites qualified individuals to apply for the position of Chair of the Department of Pharmacological and Pharmaceutical Sciences. The department is co-located on the University of Houston main campus and the world-renowned Texas Medical Center. Current faculty research interests include signal transduction, drug delivery, transcellular transport and metabolism, cardiovascular and renal pharmacology, respiratory pharmacology, neuroscience, regulation of membrane transporters, GPCR structural proteomics, receptor cell biology, and natural products. For a detailed description of individual faculty research interests, please visit www.uh.edu/pharmacy. Department faculty members contribute to the Pharm.D., Ph.D. and Pharm.D./Ph.D. degree programs. Candidates must show strong evidence of academic leadership, a commitment to professional and graduate education and peer recognition as an accomplished researcher. Successful candidate is expected to have a nationally recognized and funded research program that would complement existing research programs within the department. The new Chairperson will have faculty lines to fill, and should have the ability to recruit and develop outstanding faculty. The successful candidate also may be eligible to receive one of several Endowed Professorships.

Applications will be reviewed until the position is filled. Candidates should send a letter describing research interests, administrative and educational philosophies and long-term goals, a curriculum vitae and the names, addresses (postal and e-mail), phone and fax numbers of at least three professional references in electronic (PDF) format to:

Mustafa Lokhandwala, Ph.D., Chair, Search Committee
C/O_salazar@uh.edu
University of Houston College of Pharmacy
Houston, TX 77204-5000

The University of Houston is an Affirmative Action/Equal Opportunity Employer. Minorities, women, veterans, and persons with disabilities are encouraged to apply.



The University of New Mexico

Faculty Position in the Immunological or Inflammatory Basis of Neurological Disorders

The Departments of Molecular Genetics and Microbiology and Neurology at the University of New Mexico School of Medicine (<http://hsc.unm.edu/som/micro/>) are seeking a faculty member with experience and interest in studying immunological aspects of neurological disorders to participate in a new Brain and Behavior Illness Institute. Examples of desired areas of research include, but are not limited to:

- Innate immunity and inflammation in stroke
- Autoimmune diseases of the central or peripheral nervous system
- Migration of immune cells through the blood brain barrier
- Immunological aspects of Alzheimer's Disease and other neurodegenerative disorders
- Immunological approaches to infectious diseases of the central nervous system
- Etiology and treatment of other neurological disorders with immunological components
- Mechanisms of cell death and survival in neurological disorders

The successful applicant will have the opportunity to pursue interests in immunology, neurobiology, and translational research, using multi-disciplinary approaches. Appointment will be at the rank of Assistant Professor with a primary appointment in the Department of Molecular Genetics and Microbiology and a joint appointment in the Department of Neurology. She or he will be expected to develop a research program in immunology or inflammation of neurological disorders, including but not limited to multiple sclerosis and other immune etiology disorders, and inflammatory processes in cerebral ischemia and neurodegenerative disorders. Neurosciences are a signature program in the UNM School of Medicine and interaction with other signature programs are also highly encouraged and anticipated. There are opportunities for utilization of animal and human MRI, 2-photon microscopy, and electron paramagnetic resonance in the NIH P30-sponsored Brain Imaging Center. A very strong interaction with other members of the two departments and with other faculty engaged in the study of neuroinflammation is expected. The faculty member will participate in teaching medical students, graduate students and neurology trainees and in departmental and institutional activities.

Minimum Requirements:

- Ph.D., M.D. or equivalent
- 2 years of postgraduate research experience in neuroinflammation, inflammation, or cerebral ischemia
- eligible to work in the United States

Desirable Qualifications:

- strong record of scientific accomplishments
- high probability of receiving external funding
- potential for research and educational interactions with members of the Department of Molecular Genetics and Microbiology and Neurology and the Neuroinflammation Group
- potential synergy with UNM Health Sciences Center BBI and other signature programs <http://hsc.unm.edu/research/info/programs.shtml>
- potential synergy with UNM Clinical and Translational Science Center <http://hsc.unm.edu/research/ctsc/index.shtml>

UNM places a high priority on the success of its faculty. The successful applicant will be given protected time and mentoring by senior faculty to increase the likelihood of success in extramural funding.

For best consideration apply by, **November 12, 2010**.

The position will remain open until filled.

For complete details of this position or to apply, please visit this website: <https://unmjobs.unm.edu>. Please reference posting Number: **0808254**. For additional information you may contact: **Dolores Tarin, Search Coordinator, (505) 272-5249, dtarin@salud.unm.edu**.

UNM is an Equal Opportunity/Affirmative Action Employer and Educator.

The University of New Mexico's confidentiality policy (Disclosure of Information about Candidates for Employment, UNM Board of Regents' Policy Manual 6.7), which includes information about public disclosure of documents submitted by applicants, is located at <http://www.unm.edu/~bipm/r67.htm>.



**THOMPSON CHAIR of
Invertebrate Paleontology
Florida Museum of Natural History
University of Florida**

The Florida Museum of Natural History, University of Florida, invites applications for the Thompson Chair of Invertebrate Paleontology to be hired at the level of Associate or Full Curator (equivalent to Associate or Full Professor) with tenure. The successful candidate will be expected to conduct a dynamic research program and develop the museum's extensive collection of primarily Cenozoic invertebrate fossils that includes five million specimens. The collection is assigned two full-time staff responsible for its operations. A substantial endowment is associated with this position.

This position requires a strong commitment to university education, field work, museum-based research, and outreach. Interactions with allied academic departments include affiliate professorship status with responsibility for supervision of graduate students and teaching two formal courses per year. Minimum qualifications: Strong externally funded research, collections experience, and tenure or at least seven years of post-Ph.D. professional experience (i.e., in academic, research, or related position[s]). The start date is open. The salary is competitive and commensurate with experience.

The search committee will begin reviewing applications on January 14, 2011, and will continue to receive applications until the position (requisition #0806086) is filled. To ensure full consideration please apply online at website: <http://jobs.ufl.edu>. The application should include: (1) cover letter; (2) curriculum vitae; (3) statement of research, collections, teaching, and outreach experience, vision, and goals; (4) reprints of no more than three publications; and (5) the names of three colleagues who might be contacted for letters of recommendation. Any questions regarding this position or nominations may be directed to the search committee chair: **Dr. Bruce J. MacFadden, Thompson Chair Search Committee, Florida Museum of Natural History, University of Florida, P.O. Box 117800, Gainesville, FL 32611-7800 (e-mail: bmacfadd@flmnh.ufl.edu).**

The University of Florida is an Equal Opportunity/Affirmative Action Employer. If an accommodation due to a disability is needed to apply for this position, please call 352-392-2477 or the Florida Relay System at 800-955-8771 (TDD). The selection process will be conducted under the provisions of Florida's "Government in the Sunshine" and Public Records laws.

**DISTINGUISHED POSTDOCTORAL
FELLOWSHIP at EMSL**

The EMSL William Wiley Postdoctoral Fellowship is designed to attract high performing, newly graduated, junior Ph.D. scientists with potential to become full-time scientific staff at EMSL, a Department of Energy user facility at Pacific Northwest National Laboratory.

Candidates must display superb ability in scientific research and must show promise of becoming outstanding leaders in the research they pursue, as illustrated by their application materials, to include:

- (1) A current curriculum vitae showing all research publications and experience.
- (2) A Statement of Research Interest (not a formal research proposal) that aligns with one or more EMSL science themes (website: <http://www.emsl.pnl.gov/science/>).
- (3) Copies of unofficial transcripts for all degrees.

You will need to upload one PDF file that includes the components listed above. Applications lacking these components will be declared ineligible. Apply online at website: <http://go.usa.gov/x7E>.

Fellowships are awarded for a one-year term, with possible renewal up to three years total. It carries a high starting salary, with extra allocations for research allowances and travel. Candidates will be competitively selected by an EMSL fellowship committee and must collaborate with EMSL scientists in a research area that aligns with one or more EMSL science themes (website: <http://www.emsl.pnl.gov/science/>).



The Biology Department of Middle Tennessee State University website: <http://www.mtsu.edu/biology/> seeks applicants in the areas of Molecular and/or Cell Biology and Organismal or Evolutionary Biology, for two tenure-track positions starting in August 2011. Ph.D. in biology or related field required; postdoctoral and teaching experience preferred. Appointment rank commensurate with experience. Successful candidates will show strong potential to establish externally funded research programs and mentor undergraduate and graduate students. Teaching responsibilities may include Introductory Biology, Human Anatomy and Physiology, Genetics, Biotechnology, and electives in the candidate's area of expertise. Ability to participate in Molecular Biosciences (website: <http://www.mtsu.edu/graduate/mbsphd/>) and/or Forensic Science (website: <http://www.mtsu.edu/forensicscience/>) programs is essential.

MOLECULAR and/or CELL BIOLOGIST—Position #103060, **Dr. Jeff Leblond, Committee Chair, Box 60, MTSU, Murfreesboro TN 37132.** Additional Information: **e-mail: jleblond@mtsu.edu.**

ORGANISMAL or EVOLUTIONARY BIOLOGIST—Position #103190, **Dr. Ryan R. Otter, Committee Chair, Box 60, MTSU, Murfreesboro TN 37132.** Additional Information: **e-mail: rrotter@mtsu.edu.**

Apply at website: <http://www.mtsujobs.mtsu.edu>. A complete application includes cover letter, employment application, undergraduate and graduate transcripts, curriculum vitae, and statements of research and teaching philosophy and interests. Review of applications begins 1 November 2010. MTSU, the largest university in the Tennessee Board of Regents system, is an Equal Opportunity Employer.

ENVIRONMENTAL CHEMIST

The Department of Chemistry (website: <http://www.chemistry.appstate.edu/>) and the Environmental Science Program (website: <http://www.environment.appstate.edu/>) at Appalachian State University seek a tenure-track Environmental Chemist at the ASSISTANT PROFESSOR level beginning August 2011. Candidates with a Ph.D. in Chemistry or related field with at least 18 semester hours of graduate chemistry or applied chemistry credits will be considered. Teaching will include introductory courses in chemistry and upper division courses in chemistry and environmental science. A highly interdisciplinary background with significant research experience in environmental chemistry and a broad knowledge of earth and environmental systems is required. We prefer candidates with an ability to understand and articulate important environmental chemistry issues relevant to the Southern Appalachians. The successful candidate is expected to contribute to research and advising in the Environmental Science Program.

Electronic applications (PDF format only) should include a letter of interest and qualifications, detailed curriculum vitae, copies of undergraduate and graduate transcripts, one to two page statement of teaching and research interests, and names and contacts of three references. Submit applications to: **Professor Roy C. Sidle (e-mail: envchem@appstate.edu), Director, Environmental Science Program, P.O. Box 32067, Appalachian State University, Boone, NC 28608.** Initial application review will begin 1 November 2010, and will continue until the position is filled. A criminal background check will be conducted on all finalists who are invited to campus for an interview. *Affirmative Action/Equal Employment Opportunity Employer; for more information see website: <http://www.hrs.appstate.edu/employment/index.php>.*



**35TH ANNUAL ROSENSTIEL AWARD for
Outstanding Achievement and Distinction in
Oceanographic Science**

The Division of Marine Biology and Fisheries at the University of Miami seeks nominations for the 35th Annual Award in Oceanographic Science. The Rosenstiel Award was established by the Rosenstiel Foundation and is given annually by the Rosenstiel School of Marine and Atmospheric Sciences at the University of Miami to honor a scientist in the formative years of their careers (nominees should ideally be approximately 15 years post-Ph.D.). The 2011 award will be made in the general area of Marine Biology and Fisheries, which includes biological oceanography, marine ecology, coral reef science, ecosystem and fisheries science, and physiology and toxicology of marine organisms. Further information on the Division can be found at our website: <http://www.rsmas.miami.edu/divs/mbf>.

Nomination packages should include a letter of nomination outlining the nominee's significant contributions and potential, curriculum vitae, and a minimum of two supporting letters from persons not currently or recently associated with the nominee's institution of graduate study or employment. Please forward your nominations by November 30, 2010, to: **Dr. Su Sponaugle, Chair, Rosenstiel Award Committee, RSMAS/MBF, University of Miami, 4600 Rickenbacker Causeway, Miami, FL 33149.** Nomination packages may be submitted electronically to e-mail: shartley@rsmas.miami.edu.

POSITIONS OPEN

**ASSISTANT PROFESSOR
Utah State University**

The Department of Chemistry and Biochemistry at Utah State University invites applications for a tenure-track position at the Assistant Professor level beginning fall 2011. Candidates must have a Ph.D. in Chemistry or Biochemistry or a related field; postdoctoral experience or equivalent is preferred. The position requires the development of an externally funded research program and teaching at the undergraduate and graduate levels. Investigators with interests in the areas of Energy and the Environment, Medicinal and Synthetic Chemistry, Macromolecular Structure/Function, or Catalysis and Reaction Mechanisms, broadly defined, are strongly encouraged to apply. Applicants should submit curriculum vita, concise descriptions of future research projects and associated major research infrastructure needs, a statement concerning preferred courses to teach at the undergraduate and graduate levels, and the names and e-mail addresses of three references on-line at website: <http://jobs.usu.edu> (REQ ID 052337). Evaluation of applications will begin November 15 and will continue until the position is filled. For further information, please visit our website: <http://www.chem.usu.edu>. *Utah State University is an Equal Opportunity/Affirmative Action Employer committed to assembling a diverse faculty. Women and members of minority groups are strongly encouraged to apply.*

HARVARD MEDICAL SCHOOL

POSTDOCTORAL POSITION available to study the molecular and cellular mechanisms of Alzheimer's and Parkinson's diseases (*Nature* 460:632-636, 2009; *PNAS* 107:9879-84, 2010). Recent Ph.D.s with strong background in molecular biology, biochemistry, or electrophysiology are encouraged to apply. Prior experience in slice physiology, calcium imaging, mitochondrial analysis, or cell death mechanisms is preferred. Send curriculum vitae to **Dr. Jie Shen (website: <http://www.shenlab.net>)** at e-mail: jshen@rics.bwh.harvard.edu.

RESEARCH ASSISTANT III, protein experience required. Send resume to **Dr. H. Brunengraber, Case Western Reserve University, Department of Nutrition, School of Medicine-WG48, 10900 Euclid Avenue, Cleveland, OH 44106.** Must reference job code FF1010. *Equal Opportunity Employer.*



Assistant Professor —Tenure-Track Bioinformatics or Systems Biology San Francisco State University

We seek candidates with leadership in developing computational or quantitative methods to study biological questions, especially utilizing genomic, transcriptomic, or other highly quantitative approaches. Preference will be given to individuals whose interests complement existing departmental research strengths in molecular biology, genetics, physiology, ecology, and evolutionary biology. Responsibilities include teaching an upper-division course in bioinformatics or systems biology, programming, or other quantitative methodology. Candidates are encouraged to establish collaborative work with individuals within biology, computer science, mathematics or other departments.

Qualifications are a Ph.D. degree and postdoctoral training. Teaching experience desirable. Candidates must be committed to teaching, mentoring undergraduate and graduate (MS) students, and developing a competitive, externally-funded research program. Send cover letter, curriculum vitae, separate statements of research and teaching interests, copies of significant publications, and arrange to have three reference letters sent to: **Chair, Bioinformatics & Systems Biology Search Committee, Department of Biology, San Francisco State University, 1600 Holloway Ave., San Francisco, CA 94132-1722.** We strongly encourage electronic submission of applications as a single PDF (excluding reference letters; publications may be sent as separate PDF files) with **"Bioinformatics & Systems Biology Search"** in the subject line to biology@sfsu.edu. Review of applications begins **15 November 2010** and continues until a suitable candidate is chosen. For additional information, visit our web site at <http://biology.sfsu.edu>. SFSU and the Department of Biology are committed to a diverse professoriate that includes women and individuals from underrepresented minority groups.

SFSU is an EEO/AA Employer.



DIRECTOR SINGAPORE BOTANIC GARDENS

Established in 1859 at its present location, the Singapore Botanic Gardens (SBG) played an important historical role in the research on many tropical plants of economic value. An outstanding success was with the Para rubber tree that changed the economic and social landscape of the region. The Gardens also led in the documentation and research on tropical flora, particularly that of the Malay Peninsula. In recent decades, the Gardens embarked on a programme that expanded its plant collections; developed themed horticultural attractions; rejuvenated its botanical research, conservation and education roles; as well as provided excellent visitor amenities and services. Today, the 63.7-hectare Gardens is a key regional park, an important tourist destination as well as a well-loved civic and heritage space in Singapore. It is well connected to other botanical institutions and gardens in the region and globally. The Gardens is generally regarded as one of the best tropical botanical gardens in the world, with an average of 3 million visitors per year.

In 2008, the Gardens was awarded the Michelin three-star rating. In the same year, it was selected by Time Magazine as Asia's 'Best Urban Jungle'.

The SBG is a part of the National Parks Board, a Statutory Board of the Ministry of National Development, Singapore. The Gardens is positioned to take its place among the top botanical gardens in the world. We are seeking a dynamic professional to lead the Gardens as the Director; to develop it into a major centre for botanical research, conservation and education with a focus on the flora of the region.

The candidate will play a leading role in the management and development of the SBG into a premier global botanic gardens. The candidate is expected to cover several broad areas of responsibilities as follows:

**Collections • Botanical Research • Education
Conservation • Development
Resource Management • Recreation and Business**

Candidates may visit www.nparks.gov.sg/career for more detailed information, and apply online or submit resume to valerie_lee@nparks.gov.sg by **31 October 2010**.



Chair of the Department of Pharmaceutical Sciences and Experimental Therapeutics The University of Iowa College of Pharmacy

The University of Iowa College of Pharmacy seeks candidates for the position of Chair of the Department of Pharmaceutical Sciences and Experimental Therapeutics. The College of Pharmacy [www.pharmacy.uiowa.edu] is an important member of a comprehensive health sciences campus within a major research university. The chair will hire and mentor new faculty, stimulate new research and grant submissions, provide fiscal oversight and management, oversee faculty space utilization and teaching assignments in the Pharm.D. and graduate programs. The individual will be responsible for conducting the annual review of faculty, and organize and participate in promotions.

This position offers broader opportunities for involvement in the University research enterprise and is supported by an endowed chair in the College of Pharmacy.

The Department consists of 20 tenure track faculty members with expertise in medicinal and natural products chemistry, pharmaceuticals, pharmacokinetics, and clinical pharmacology. The faculty members have very strong scholarly programs that have substantial support from external funding. The Department is home to three Ph.D. sub-programs focused in clinical pharmaceutical sciences, medicinal and natural products chemistry and pharmaceuticals, that have more than 70 graduate students in track along with 10 post-doctoral fellows and visiting scientists. Affiliations exist with the University of Iowa Pharmaceuticals (an FDA licensed manufacturing center), the UI Center for Biocatalysis and Bioprocessing, Institute for Clinical and Translational Science, Holden Comprehensive Cancer Center, Nanoscience and Nanotechnology Institute, Environmental Health Sciences Research Center, Center on Aging and many academic programs within and outside the University.

Candidates must have a strong scientific record supported with significant extramural funding and publications, along with evidence of success in teaching and service consistent with appointment as a tenured Professor. The research area should complement existing strengths in drug discovery and development. Candidates should have experience with administrative and fiscal leadership, excellent interpersonal skills, and a strong, well recognized program of research.

The University of Iowa is located in Iowa City, a vibrant community located in southeastern Iowa, offering outstanding schools, quality entertainment, sports, literary, musical and cultural opportunities.

Review of applications will begin **November 15, 2010** and continue until the position is filled. A complete application will include a letter of interest, a complete curriculum vitae and complete contact information for three references. For further information and to apply, visit our website at <http://jobs.uiowa.edu/> Search for requisition **#58412**. For additional information please contact:

**Lawrence Fleckenstein, Pharm.D.
Professor and
Chair, Pharmaceutical Sciences and
Experimental Therapeutics
Search Committee
118 PHAR, College of Pharmacy
The University of Iowa
Iowa City, Iowa 52242
l-fleckenstein@uiowa.edu
319-335-8804**

The University of Iowa is an Equal Opportunity and Affirmative Action Employer. Women and minorities are strongly encouraged to apply.

POSITIONS OPEN



HARVARD
**School of Engineering
and Applied Sciences**

TENURE-TRACK FACULTY POSITION in Theoretical or computational earth, climate, or environmental science and engineering

The School of Engineering and Applied Sciences (SEAS) at Harvard University seeks theoretically or computationally oriented candidates in the fields of Earth Science, Climate Science, and Environmental Science and Engineering who would contribute to the intellectual and educational growth of the applied math and computational science community in SEAS. The "grand challenge" problems of this search include understanding and predicting earthquakes; modeling groundwater or surface flows and transport of contaminants; understanding and predicting climate (including theory and simulation of ocean, atmosphere, and cryosphere dynamics); and modeling, monitoring, and predicting the chemistry and biogeochemistry of the atmosphere and oceans. We are seeking candidates of exceptional scientific talent who work in one or more of these areas and who would be eager to participate in the Applied Mathematics curriculum in SEAS in addition to the environmental subject areas. The intention is to make this appointment at the untenured assistant professor level, but candidates at the untenured associate or full tenured professor level will be considered in exceptional circumstances. A strong doctoral record including demonstrated excellence in teaching is required. Send a cover letter, curriculum vitae, research statement, teaching statement, and three letters of recommendation as one PDF to **e-mail: theocompsearch@seas.harvard.edu**. Please include two to three completed manuscripts. Computer animations or similar research products are also encouraged. Applications will be accepted until the position is filled. *Applications from women and underrepresented minority candidates are strongly encouraged. Harvard University is an Equal Opportunity/Affirmative Action Employer.*

ASSISTANT/ASSOCIATE PROFESSORS Center for Phage Technology Texas A&M

The Departments of Biochemistry and Biophysics, Plant Pathology and Microbiology, and Veterinary Pathobiology at Texas A&M invite applications for tenure-track Assistant/Associate Professor positions, two to be filled for academic year 2011-12 and two more the following year. The positions are associated with a new interdisciplinary Center for Phage Technology (CPT). The CPT is dedicated to bacteriophage biology and to its potential for antibacterial strategies in human and animal medicine, agriculture, and industry. Direct experience or current activity in phage biology per se is not required, but a research program or expertise, including prokaryotic microbiology, structural biology, and bioinformatics, that would help advance the broad goals of the CPT is desirable. Successful candidates are expected to establish nationally competitive research programs and to participate fully in the educational programs of the respective departments. Competitive startup packages, salaries, and laboratory space will be provided. Please send a PDF file that contains a cover letter, curriculum vitae, research summary (past, present, and planned), and a teaching statement to **e-mail: cpr@tamu.edu**. Applications received by November 15 will be guaranteed full consideration by the Interdepartmental Search Committee, although review of applications will continue until the positions are filled. *Equal Opportunity Employer.*

The Misher College of Arts and Sciences at University of the Sciences in Philadelphia invites applications for the positions of **CHAIR**, Department of Biological Sciences, and **CHAIR**, Department of Chemistry and Biochemistry to begin July 1, 2011. The successful candidate will possess the ability to bring the departments to the next level of national recognition and achievement. For a full job description and application details, visit us at **website: http://www.usp.edu/humanresources/employment**.

Affirmative Action/Equal Opportunity Employer.

POSITIONS OPEN

FACULTY POSITIONS Pharmacology and Pharmacokinetics

The Department of Pharmaceutical Sciences at North Dakota State University invites applications for two tenure-track faculty positions (one in pharmacology and one in pharmacokinetics) at the rank of **ASSISTANT/ASSOCIATE/FULL PROFESSOR**, with appointment beginning on or after January 1, 2011. A highly competitive salary and a startup package commensurate with qualifications and experience are available. Currently, the fast growing department has 12 faculty members, 30 doctoral students, and eight postdoctoral fellows/research associates, has a Center of Excellence, Center of Biopharmaceutical Research and Production (CBRP), and participates in a NIH-funded (\$10.5 million) Center of Biomedical Research Excellence. Additional information about the Department and University can be obtained at **website: http://www.ndsu.edu/pharmsci/**.

Assistant/Associate/Full Professor in Pharmacology: Candidates must hold a doctoral degree in Pharmacology, Physiology, or closely related field, have at least two years of postdoctoral experience with a strong record of scholarship, and possess good interpersonal skills, and effective written and oral communication skills. Preference will be given to applicants with training and research expertise in areas that complement existing departmental strengths in cancer and cardiovascular research. The successful candidate will be expected to establish an externally funded research program, teach and mentor graduate students, and participate in team-taught pharmacology courses offered to pharmacy students. Application deadline is November 15, 2010, or thereafter until the position is filled.

The application portfolio containing the curriculum vitae, statement of teaching philosophy, description of research interests and future plans, and three letters of reference must be submitted electronically at **website: http://jobs.ndsu.edu/applicants/Central?quickFind=51550**. For more information, please contact **Dr. Bin Guo (e-mail: bin.guo@ndsu.edu; telephone: 701-231-5164)**, North Dakota State University College of Pharmacy, Nursing and Allied Sciences, Fargo, ND 58105.

Assistant/Associate/Full Professor in Biopharmaceutics and Pharmacokinetics: Candidates must hold a doctoral degree in biopharmaceutics or pharmacokinetics, drug metabolism, drug delivery or closely related field, have at least two years of postdoctoral experience either in biopharmaceutics/pharmacokinetics, drug delivery or drug metabolism with a strong record of scholarship, and possess good interpersonal skills and effective written and oral communication skills. Preference will be given to applicants with training and research expertise in areas of biopharmaceutics or pharmacokinetics, drug delivery or metabolism. The successful candidate will be expected to establish an externally funded research program, teach and mentor graduate students, and participate in teaching of biopharmaceutics and pharmacokinetics to Pharm.D. Professional and graduate students.

Faculty rank and salary will be dependent upon qualifications and experience.

Application deadline is November 15, 2010, or thereafter until the position is filled. The application portfolio containing the curriculum vitae, statement of teaching philosophy, description of research interests and future plans, and three letters of reference must be submitted electronically at **website: http://jobs.ndsu.edu/applicants/Central?quickFind=51546**. For more information, please contact **Dr. Bin Guo (e-mail: bin.guo@ndsu.edu; telephone: 701-231-5164)**, North Dakota State University College of Pharmacy, Nursing and Allied Sciences, Fargo, ND 58105.

NDSU is an Equal Opportunity Institution. Women and traditionally underrepresented groups are encouraged to apply.

The Department of Chemistry at The University of Chicago invites applications for the position of **SENIOR RESEARCH ASSOCIATE**. Interested candidates must apply through the University's Academic Jobs **website: http://tinyurl.com/2fskev**. To ensure full consideration, all application materials must be received by November 19, 2010. Screening will continue until the position is filled. *Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

PROFESSOR OF PRACTICE in Geographic Information Systems, Tulane University

The Department of Earth and Environmental Sciences seeks to fill a non-tenure-track, Professor of Practice position to teach courses in Geographic Information Systems (GIS), and to supervise the GIS computer lab. To be considered, the applicant must have experience using geologic, topographic, and geophysical raster and point cloud data sets. Expertise with ESRI products is required, including customization of GIS applications. We seek an individual possessing an enthusiastic dedication to teaching who is willing to make a long-term commitment to the department and the University. A Ph.D. is required at the time of appointment. The initial appointment will be for three years with the possibility of renewal after a performance review at the end of the second year. The deadline for applications is October 30, 2010, but the position will remain open until filled. Applications should include curriculum vitae, a statement of teaching interests and goals, and the names and contact information of at least three references, and should be sent to: **Dr. Stephen Nelson, Department of Earth & Environmental Sciences, Tulane University, 6823 Street Charles Avenue, New Orleans, LA 70118-5698. E-mail: snelson@tulane.edu** preferred. Further information about the department and University can be obtained at **website: http://tulane.edu/sse/cens**. *Tulane University is an Affirmative Action/Equal Opportunity Employer. Women and minorities are encouraged to apply.*

EXPERIMENTAL BIOLOGICAL PHYSICS Northeastern University, Boston, MA

The Department of Physics at Northeastern University invites applications for a tenure-track position in experimental biological physics to begin September 2011. Senior appointments at the tenured level are also possible for individuals who have a demonstrated track record of outstanding research in this area. The department already has an established program in both experimental and theoretical biological physics and it is building on this base to further expand its interdisciplinary research potential across both departmental and college boundaries. The successful candidate must have a Ph.D. in Physics or a related area and is expected to establish (or have) an independent, externally funded research program and to teach effectively at undergraduate as well as graduate levels. Application materials should include curriculum vitae and a description of the candidate's research interests, as well as at least three letters of recommendation. To apply, please visit **Careers at Northeastern at website: https://pssoft.nyu.edu/psc/neuhrprdpub/EMPLOYEE/HRMS/c/NEU_HR_NEU_JOBS.GBL**, and click on Faculty Positions.

Northeastern University is an Affirmative Action/Equal Opportunity/Title IX Employer and particularly welcomes applications from minorities, women, and persons with disabilities.

SEARCH for EDITOR-IN-CHIEF The Journal of Biological Chemistry

The American Society for Biochemistry and Molecular Biology welcomes nominations for the position of Editor-in-Chief of The Journal of Biological Chemistry. The JBC publishes papers based on original research that are judged to make a novel and important contribution to understanding the molecular and cellular basis of biological processes. Candidates should have demonstrated success in research in one of the fields about which the JBC publishes and should possess: A broad, general knowledge of biological chemistry; commitment to publishing the very best science; the ability to recruit outstanding scientists to serve as contributors, associate editors and editorial board members; a willingness to provide sustained and consistent editorial direction.

Nominations should include a summary of the candidate's career and research interests. If self-nominating, the candidate should include a statement about his or her approach to leading the JBC. Nominations should be sent electronically by November 1, 2010, to **e-mail: nrodnan@asbmb.org**.

FACULTY POSITIONS BIOLOGY DEPARTMENT BOSTON COLLEGE

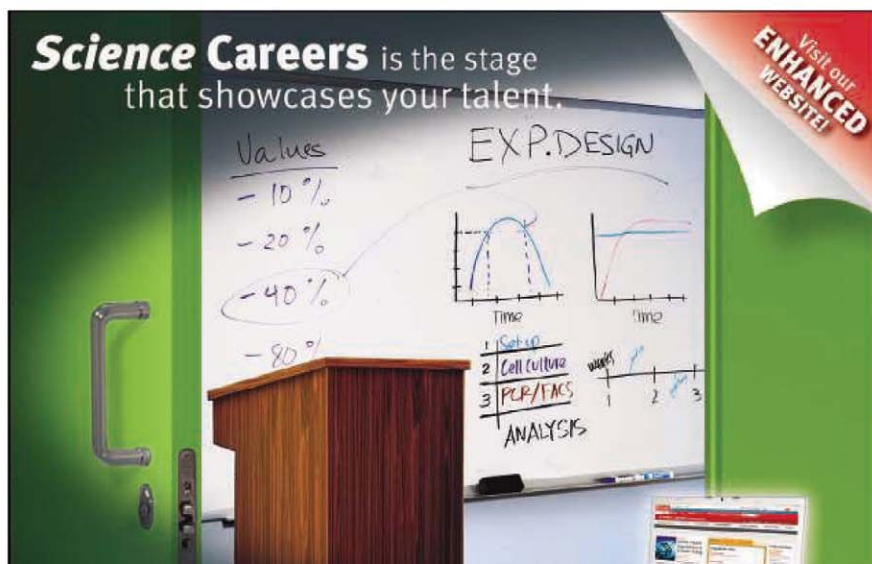
We invite applications for a tenure-track faculty position in the Boston College Biology Department, at the level of **ASSISTANT** or **ASSOCIATE PROFESSOR**. The university provides competitive startup funds and research space with the expectation that the successful candidate will establish, or bring to the university, a vigorous, externally funded research program. We seek colleagues whose research mesh with current faculty interests in molecular and cell biology, genetics and genomics, bioinformatics and computational biology, structural biology, or neuroscience (<http://www.bc.edu/biology>). Special consideration will be given to candidates with a research focus on immunology, innate immunity and infectious diseases with appropriate animal models. In addition to directing an active research program, the successful candidate will be expected to train graduate students and participate in the undergraduate teaching mission of the department. This appointment will begin on or after July 1, 2011.

Applicants should submit PDFs of their curriculum vitae, statement of present and future research plans, and three letters of reference to: **BCFacultySearch@gmail.com**. Applications should be received by **December 15, 2010**, to assure full consideration. Review of applications will continue until the position is filled.

*Boston College is an Affirmative Action,
Equal Opportunity Employer, committed to
improving diversity.*

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MEETINGS



CAREER CHOICES IN SCIENCE

ROYAL SOCIETY LONDON 19 NOVEMBER 2010

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POSITIONS OPEN

FACULTY POSITIONS Biology and Physics

Kennesaw State University - Tenure-track, **ASSISTANT PROFESSOR** positions in Cell Biology, Ecological Modeling, Molecular Plant Systematics and Physics will be available in the Department of Biology and Physics at Kennesaw State University beginning August 2011. An earned doctorate in an appropriate discipline is required. Applicants should have a strong potential for developing an externally funded research program as well as postdoctoral experience. Preference will be given to applicants with demonstrated excellence in teaching at the college level.

For a complete description of positions, go to **website:** <http://www.kennesaw.edu/facultypositions/>

To guarantee consideration, application materials must be postmarked by November 1, 2010.

Submit a letter describing qualifications for the position, a statement of teaching philosophy, a statement of research interests, a current curriculum vitae, graduate transcripts, and the names, addresses, telephone numbers, and e-mail addresses of three references to: **Dr. Dale Lynn Vogeliën**, Cell Biology Search Committee; **Dr. Bill Ensign**, Ecological Modeling Search Committee; **Dr. Paula Jackson**, Plant Systematics Search Committee; or **Dr. Ted LaRosa**, Physics Search Committee; at **Department of Biology & Physics, Kennesaw State University, 1000 Chastain Road, #1202, Kennesaw, GA 30144-5591.**

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FACULTY POSITION in GENOMICS at The University of Arizona

The Department of Ecology and Evolutionary Biology at the University of Arizona seeks to fill one tenure-track faculty position in the broad area of genomics and the environment, particularly with applications to human health. Candidates' research accomplishments and aims should show evidence of originality and should address significant evolutionary and/or ecological questions. Methods and approaches can include population genetics, functional and comparative genomics, bioinformatics, field and experimental studies, and can focus on any group of organisms. We are particularly interested in researchers who link genomics to the environment, for example by identifying the genetic basis of adaptation to different environments, the expansion of infectious diseases into new geographic regions as a result of climate change or changes in land use, and the environmental determinants of human genetic diseases. We will consider applicants at all ranks (**ASSISTANT, ASSOCIATE, and FULL PROFESSOR**). Prominent senior candidates are especially encouraged to apply. Review of applications will begin November 1, 2010. To apply, please submit a cover letter, a statement of research interests, a statement of teaching interests, complete curriculum vitae, up to five reprints of published work, and arrange for three letters of recommendation to be sent to: **Genomics Search Committee, Department of Ecology & Evolutionary Biology, 1041 E. Lowell Street, University of Arizona, Tucson AZ 85721.**

POSTDOCTORAL POSITION Climate/Energy Decision Making Carnegie Mellon University

Postdoctoral position in Climate/Energy Decision Making: Prefer B.S. in science or engineering and Ph.D. in behavioral social sciences. For details, see Postdoctoral positions at **website:** <http://www.cpp.cmu.edu/jobs>. Send resume, sample papers, and list of references to Meryl Sustarsic (e-mail: meryls@andrew.cmu.edu).

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1-9. *Science*, Publication No. 0036-8075, is published weekly on Friday, except the last week in December, at 1200 New York Avenue, N.W., Washington, DC 20005. Date of filing: 27 September 2010. This is also the address of the publisher, the editor, and the managing editor, who are, respectively, Beth Rosner, Bruce Alberts, and Monica M. Bradford.

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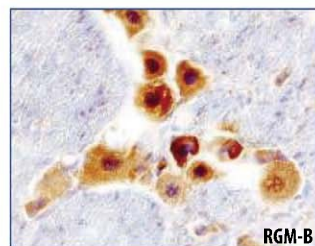
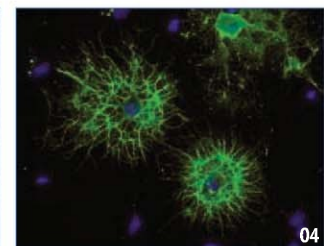
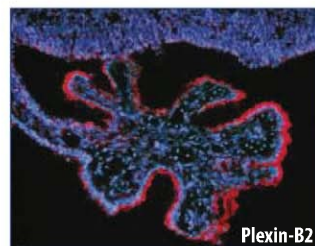
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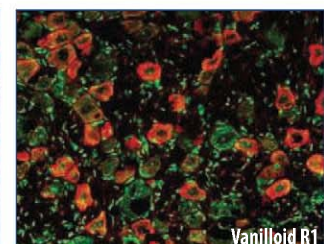
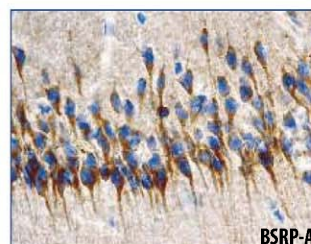
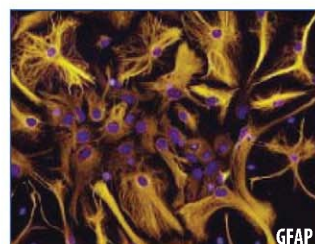
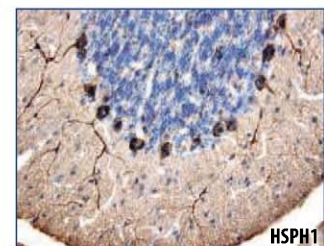
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